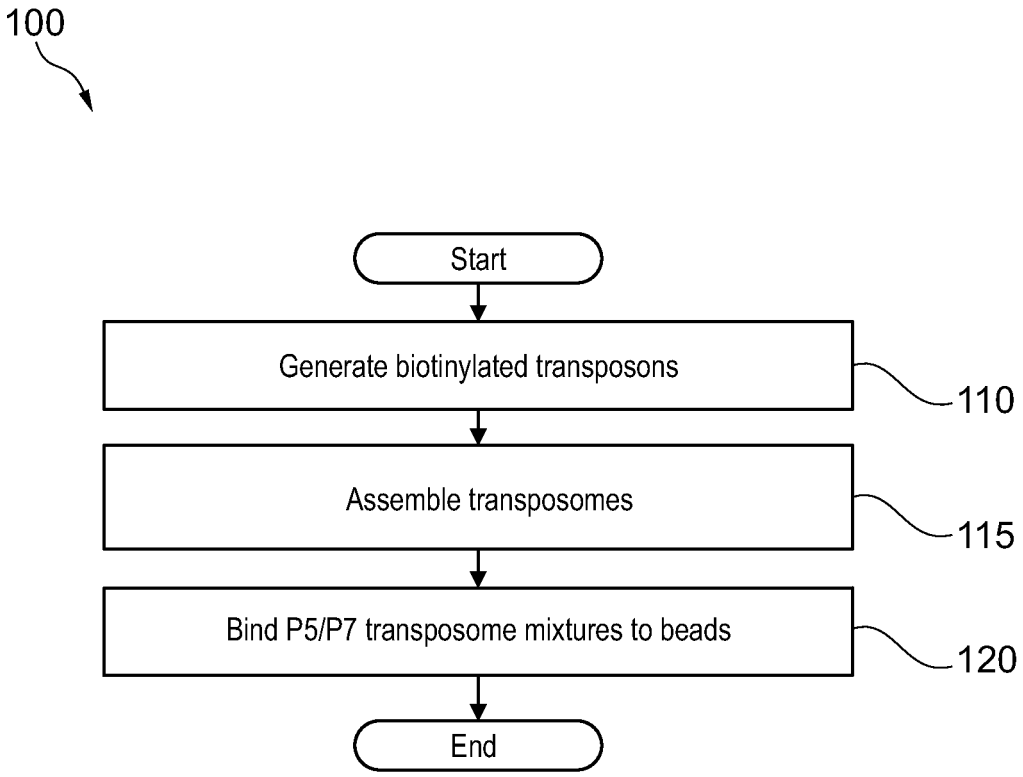

100
Start
Generate biotinylated transposons
110
Assemble transposomes
115
Bind P5/P7 transposome mixtures to beads
120
End

Fig. 1

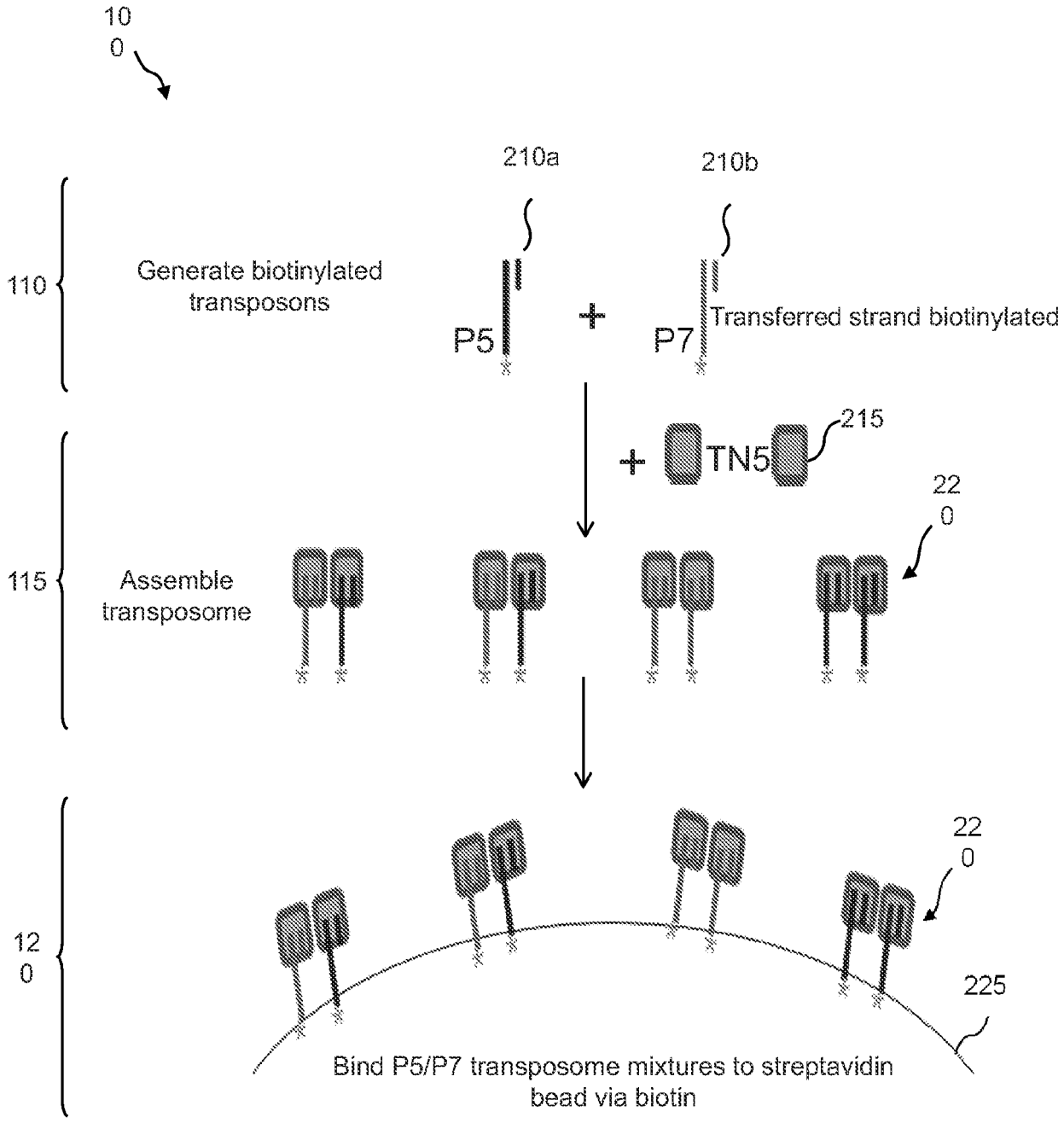

10
0
210a
210b
110
Generate biotinylated transposons
P5
+
P7
Transferred strand biotinylated
215
+
TN5
22
0
115
Assemble transposome
12
0
22
0
225
Bind P5/P7 transposome mixtures to streptavidin bead via biotin

Figure 2

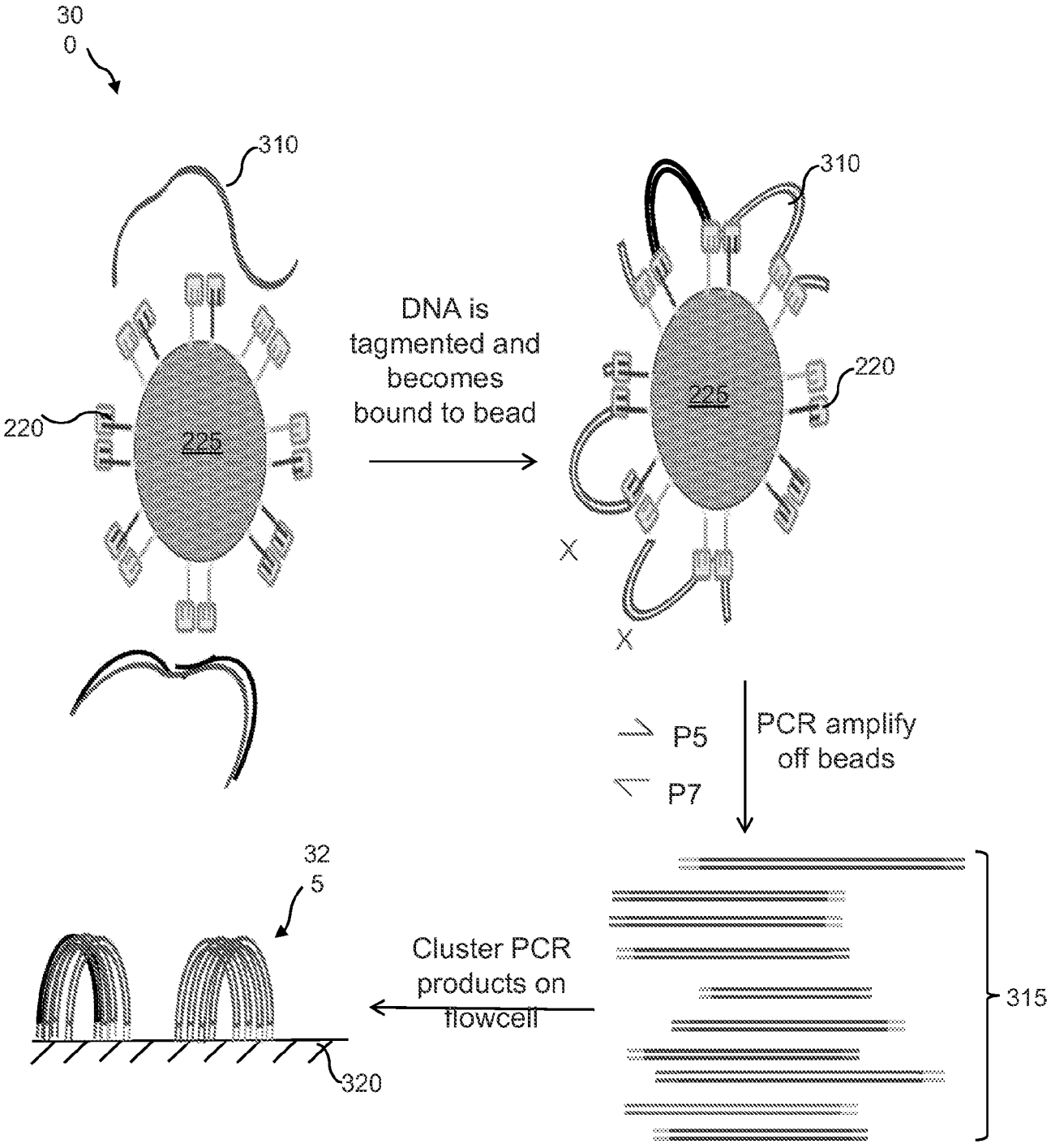

30
0
310
220
225
DNA is
tagmented and
becomes
bound to bead
310
220
225
X
X
P5
P7
PCR amplify
off beads
315
32
5
Cluster PCR
products on
flowcell
320

Figure 3

400

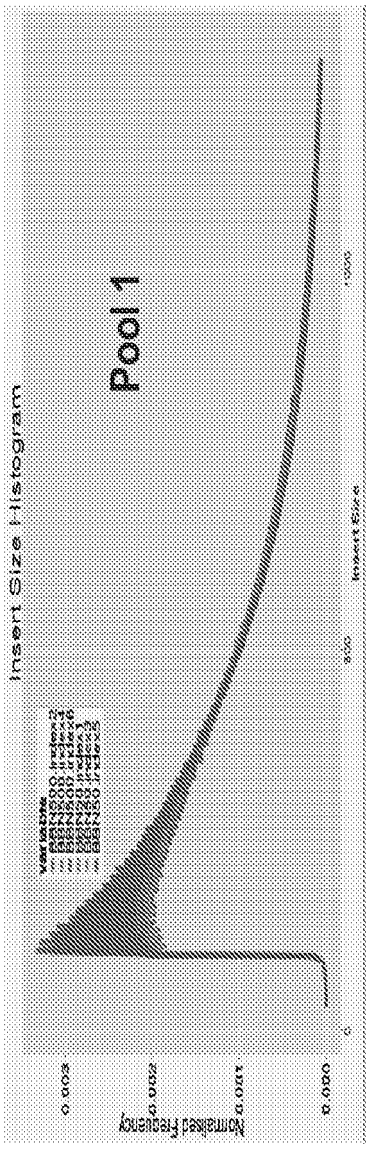
Sample	No of clusters with equal volume of PCR product							
pM used to cluster	15		19		21		24	
	K/mm2	%	K/mm2	%	K/mm2	%	K/mm2	%
50 ng	466	100	541	100	618	100	668	100
250ng	504	108	598	110	703	114	740	111
1000ng	512	109	602	111	670	108	716	107
*50ng full repeat	377	81	455	84	516	83	555	83

Figure 4

500

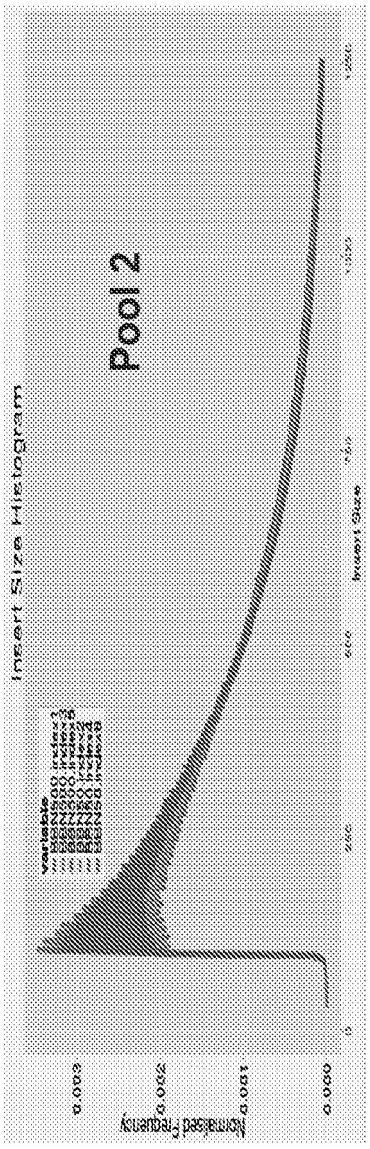
	Median Insert Size	Mean Insert size
BBNS0-index1	341	409.78
BBNS00-index1	344	415.89
BBNS0-index2	337	405.97
BBNS00-index2	346	419.91
BBNS0-index3	330	400.35
BBNS00-index3	327	398.65
BBNS0-index4	321	388.71
BBNS00-index4	328	400.75
BBNS0-index5	327	397.62
BBNS00-index5	328	399.88
BBNS0-index6	324	389.35
BBNS00-index6	331	401.50

Figure 5



600

Figure 6A



650

Figure 6B

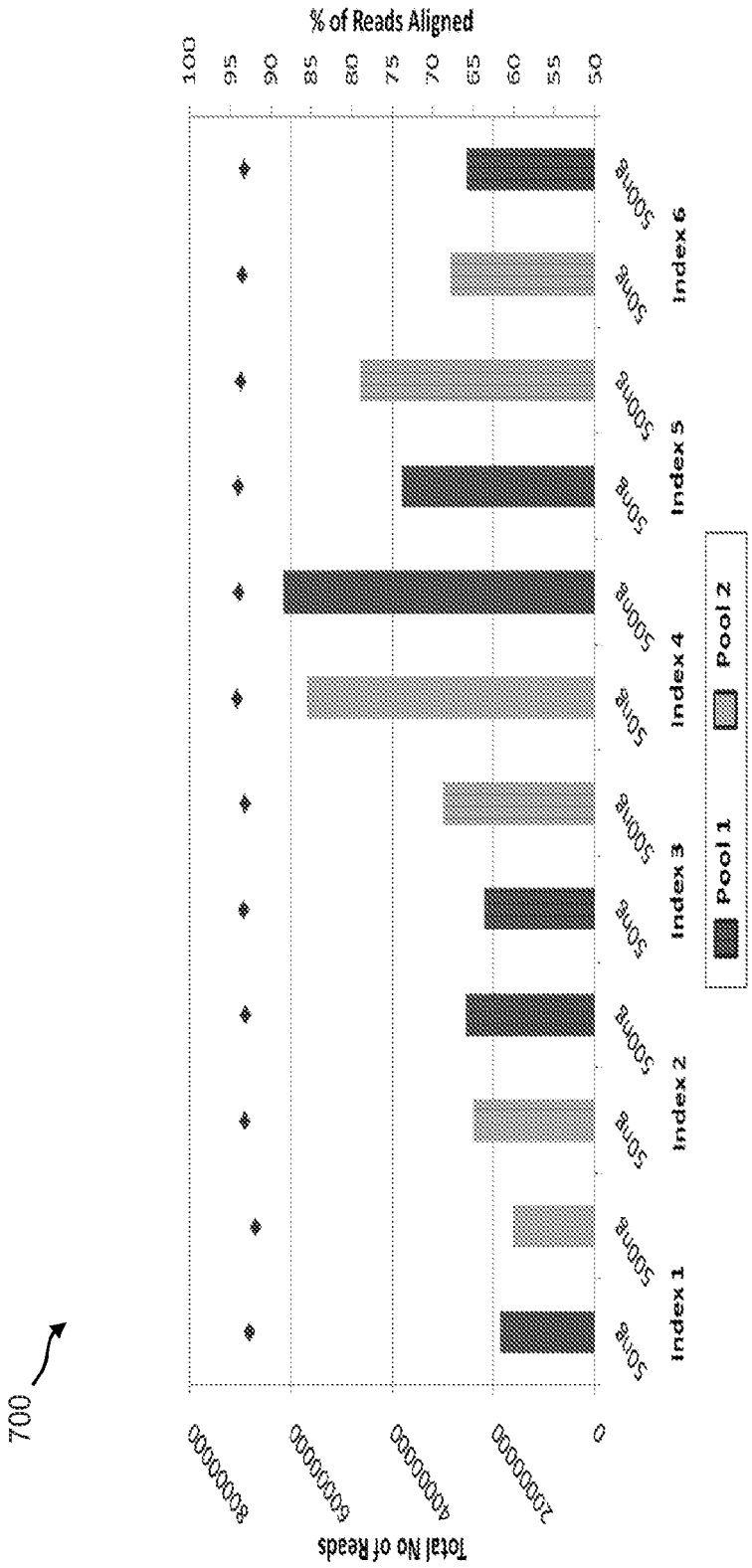

700
% of Reads Aligned
100
95
90
85
80
75
70
65
60
55
50
Total No of Reads
800000000
600000000
400000000
200000000
0
Index 1
Index 2
Index 3
Index 4
Index 5
Index 6
Pool 1
Pool 2

Figure 7

Figure 8A

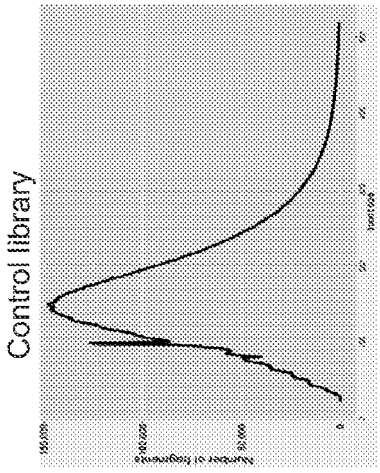
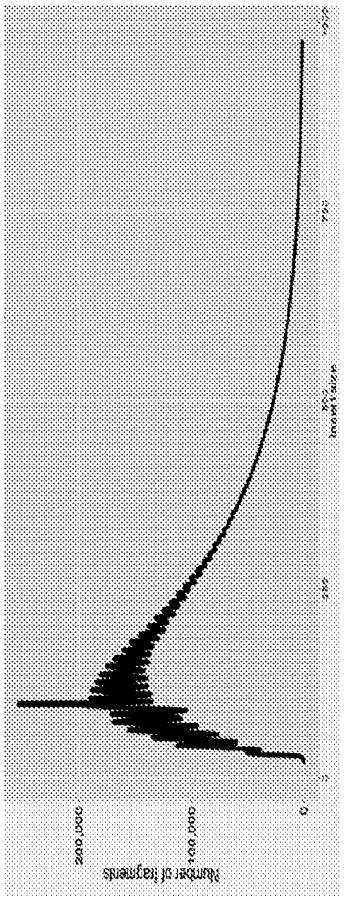


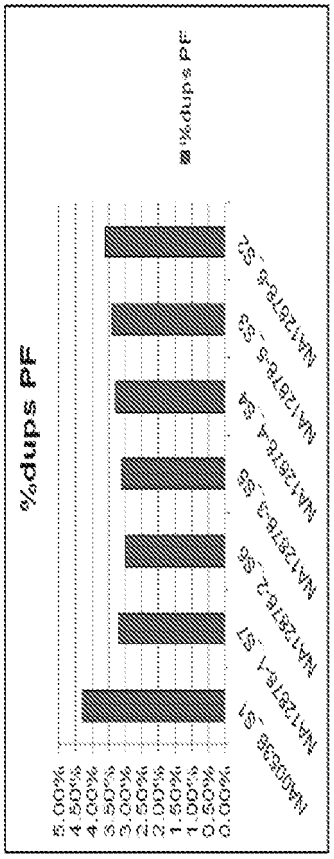
Figure 8B



840

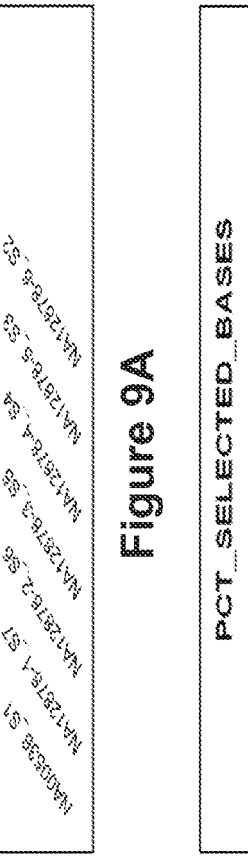
	Control	25ng	50ng	100ng	150ng	200ng	500ng
Mean	196.8	267.4	287.3	288.8	288.6	295.0	282.7
Median	163	206	222	224	223	230	220
mode	149	98	98	98	98	98	98

Figure 8C



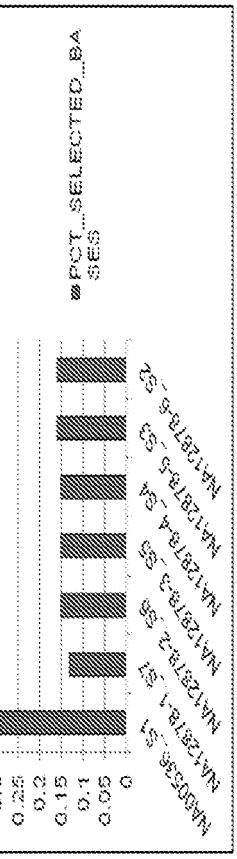
900

Figure 9A



920

Figure 9B



940

Figure 9C

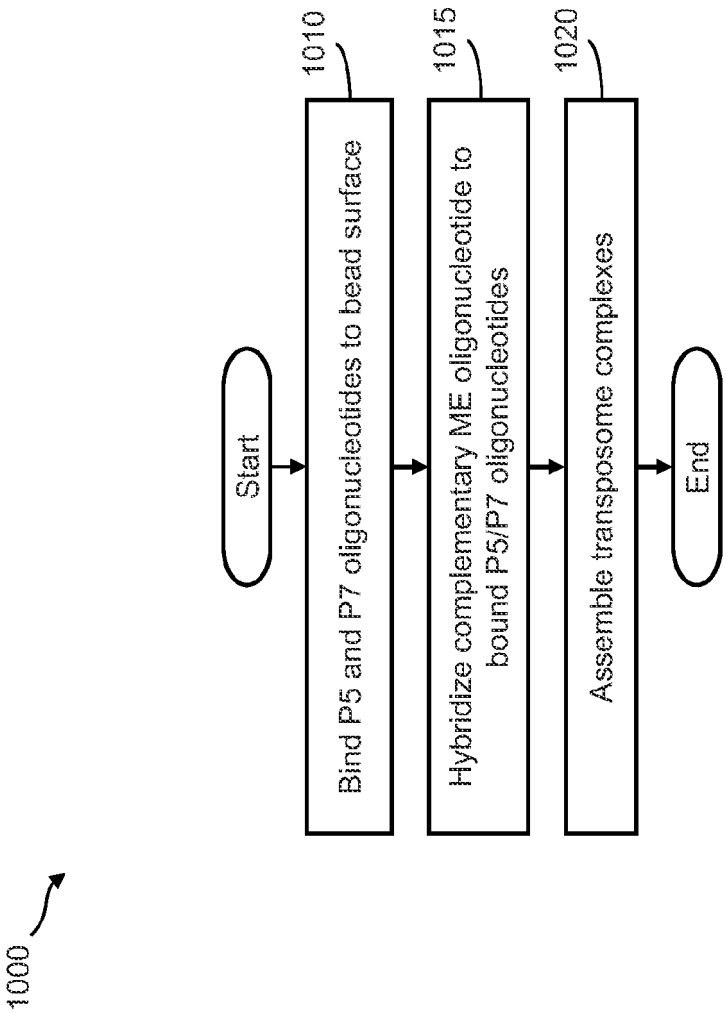

1000
Start
1010
Bind P5 and P7 oligonucleotides to bead surface
1015
Hybridize complementary ME oligonucleotide to bound P5/P7 oligonucleotides
1020
Assemble transposome complexes
End

Figure 10

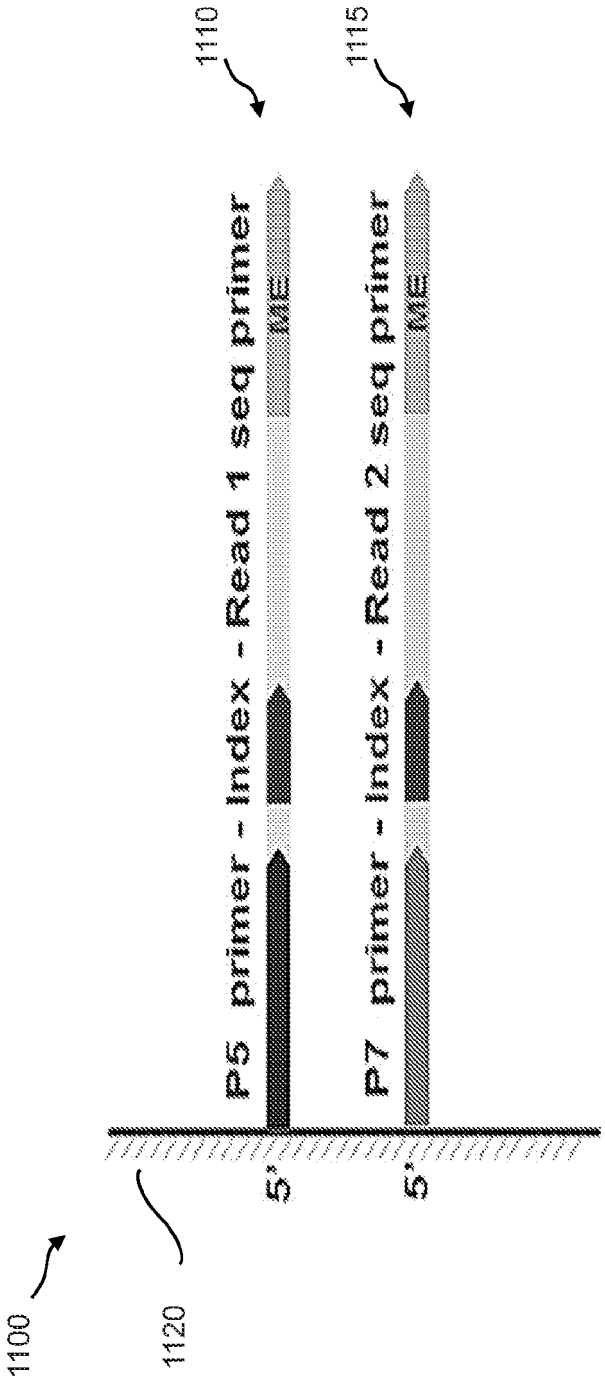

1110
1115
P5 primer - Index - Read 1 seq primer
ME
P7 primer - Index - Read 2 seq primer
ME
5'
5'
1100
1120

Figure 11

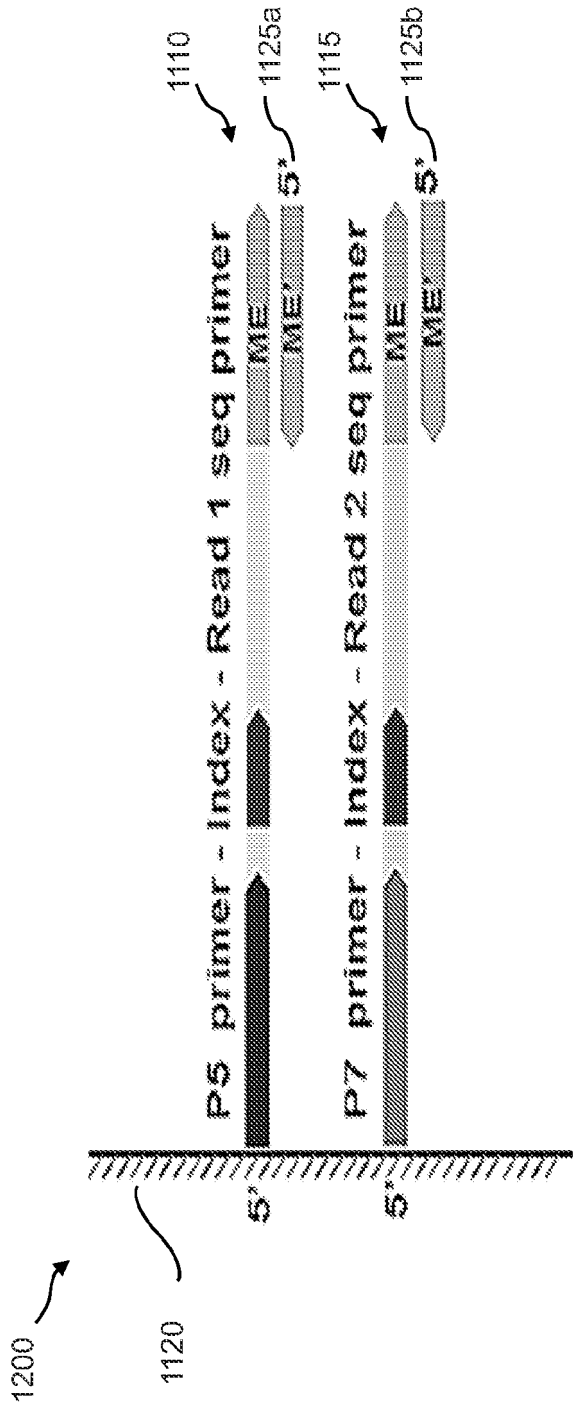

1200
1120
1110
1125a
1115
1125b
P5 primer - Index - Read 1 seq primer
P7 primer - Index - Read 2 seq primer
ME
ME'
5'
5'
5'
5'

Figure 12

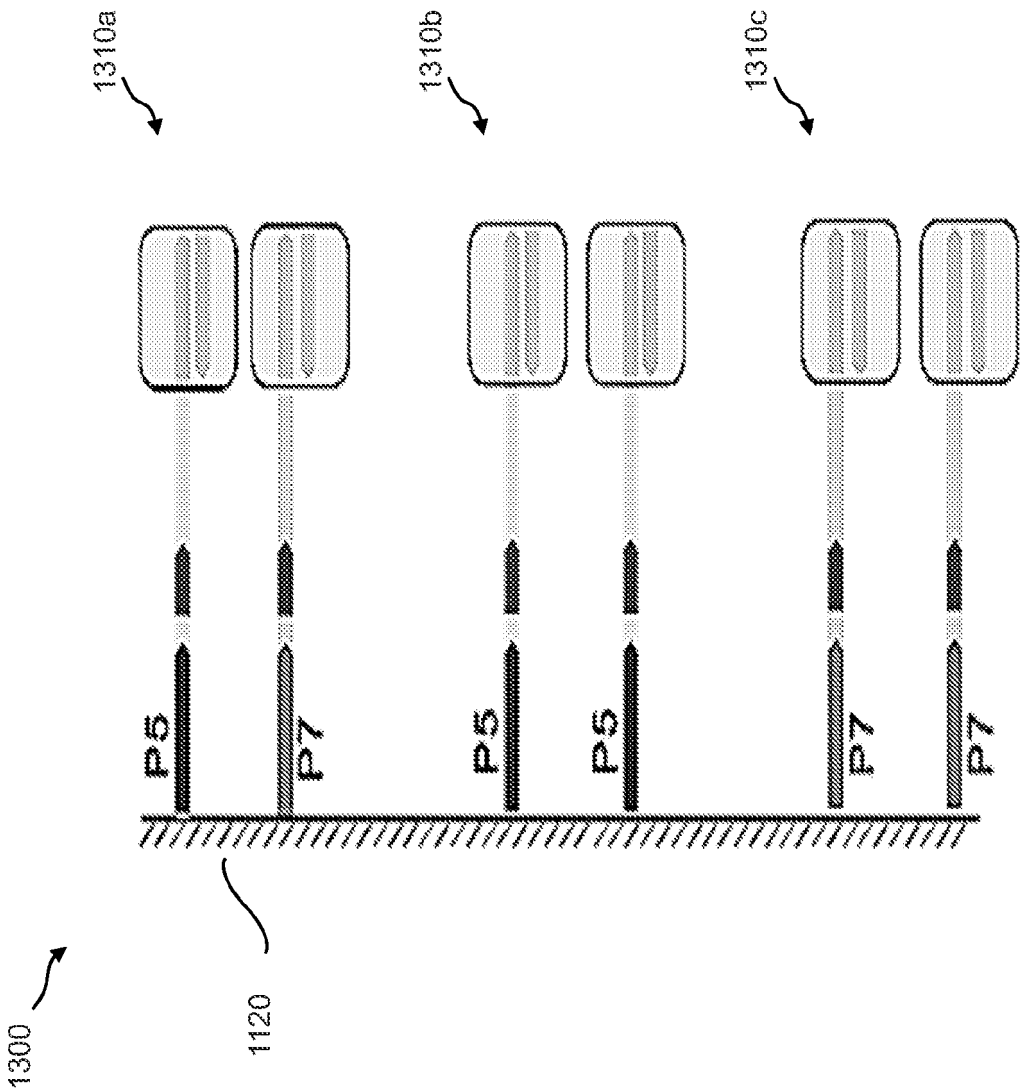


Figure 13

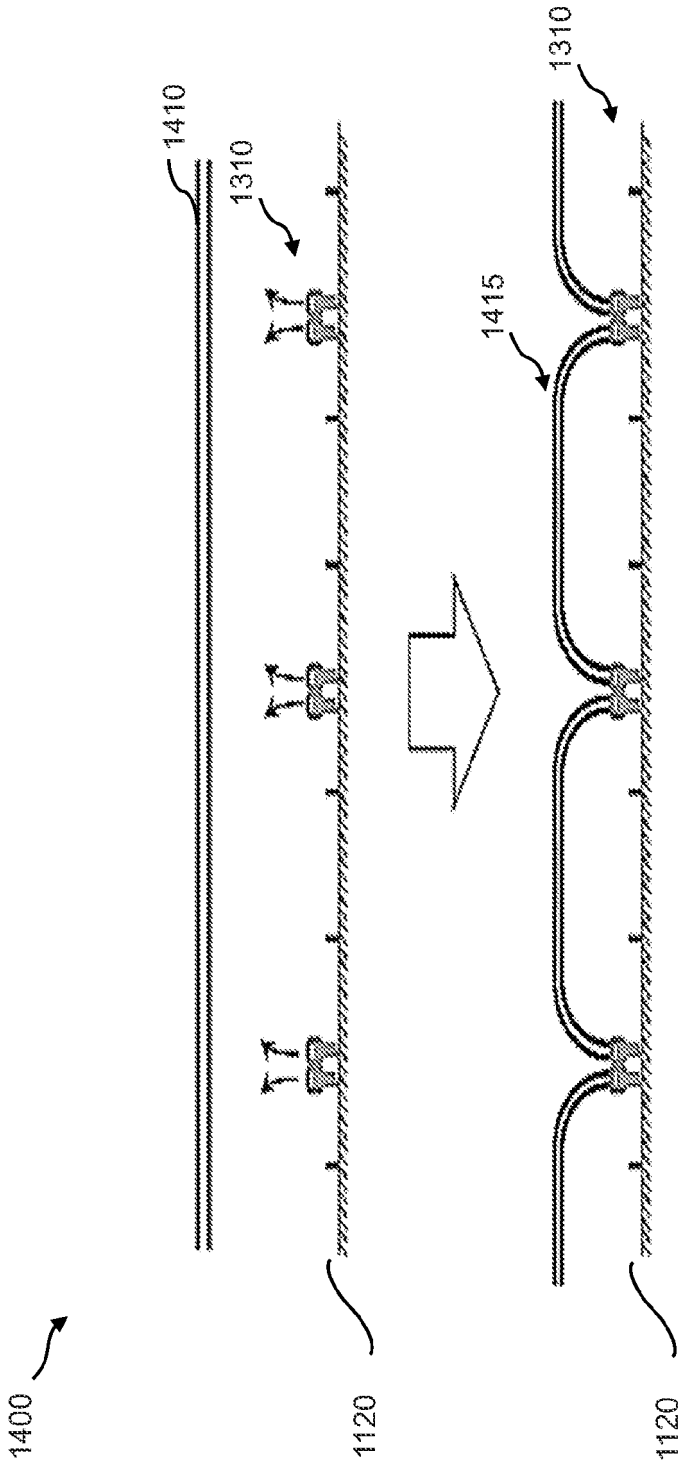


Figure 14

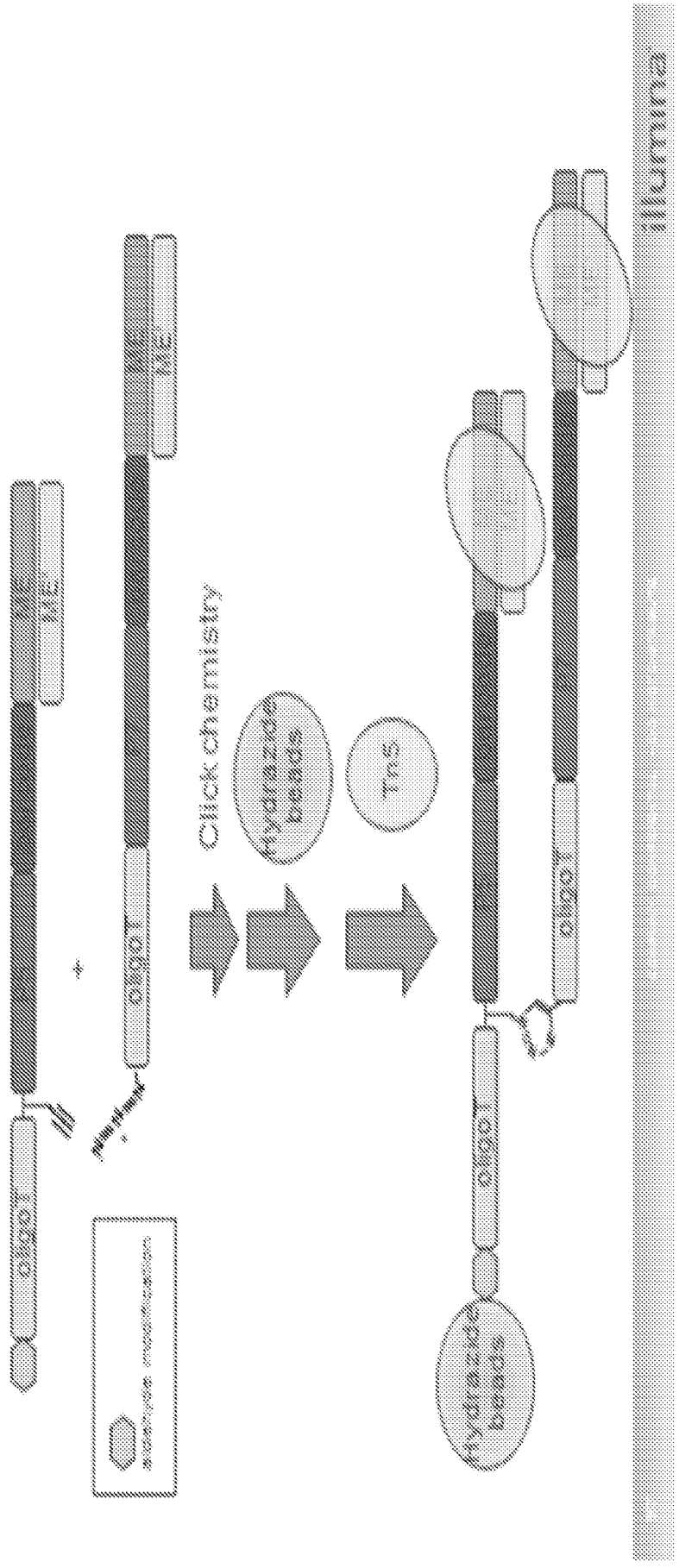

ME
ME
ME
ME
adapt
adapt
adapt
adapt
Click chemistry
hydrazide beads
Tris
hydrazide beads
Illumina

Figure 15

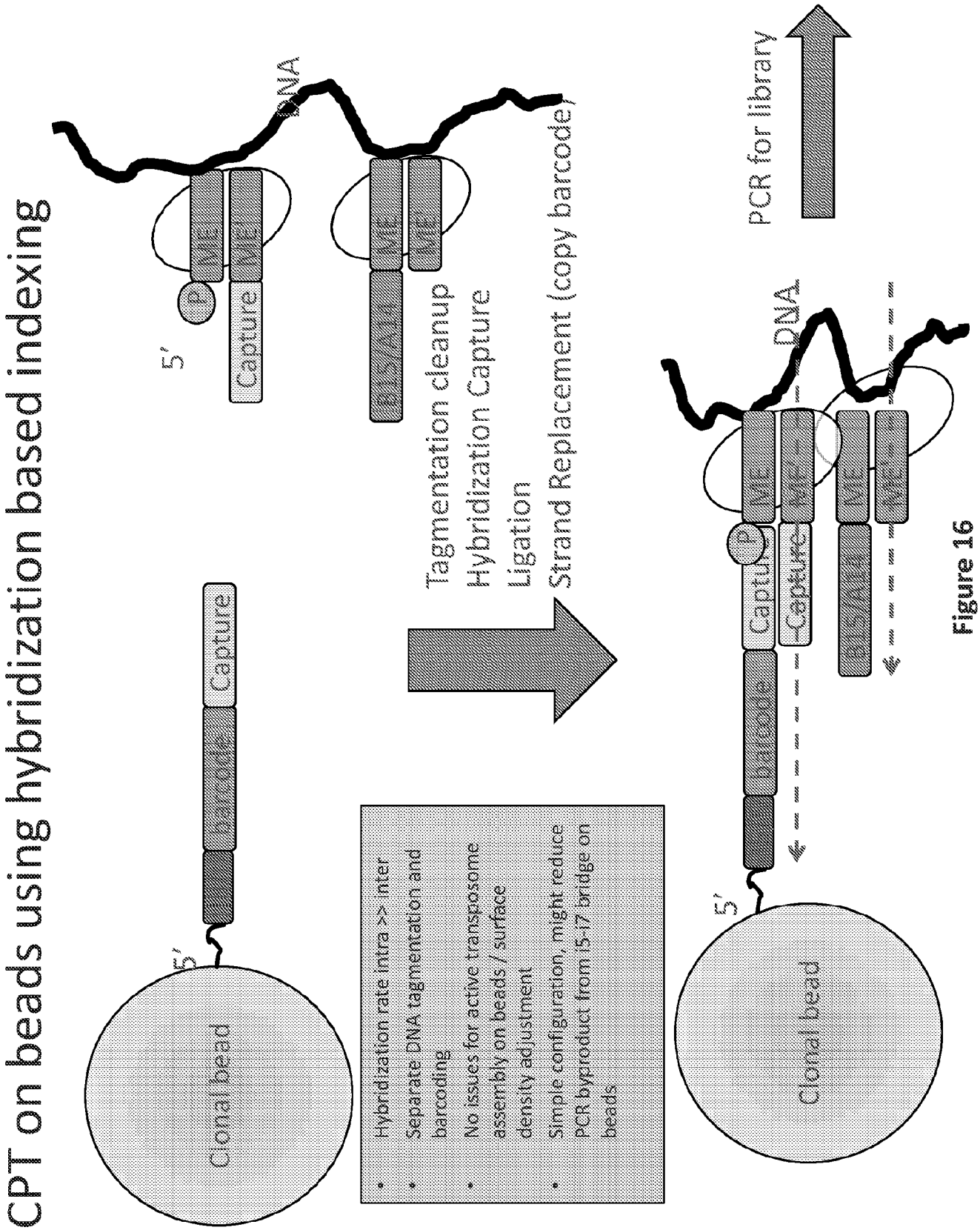

CPT on beads using hybridization based indexing
Clonal bead
5'
Barcode
Capture
Hybridization rate intra >> inter
Separate DNA tagmentation and barcoding
No issues for active transposome assembly on beads / surface density adjustment
Simple configuration, might reduce PCR byproduct from i5-i7 bridge on beads
Tagmentation cleanup
Hybridization Capture
Ligation
Strand Replacement (copy barcode)
DNA
P
ME
Capture
PCR for library

Figure 16

Contiguity Preserving Transposition (CPT) on Beads
DNA
ME
5'
P
P
A7'
A14
ME'
+
5'
M280
B15 Ext
Barcode
A7
3'
PCR with P7-index-B15 & P5-index2-A14
DNA
P7-index1-B15ext-xxxxx-A7-ME
ME'-A14'-index2'-P5' (one strand)

Figure 17

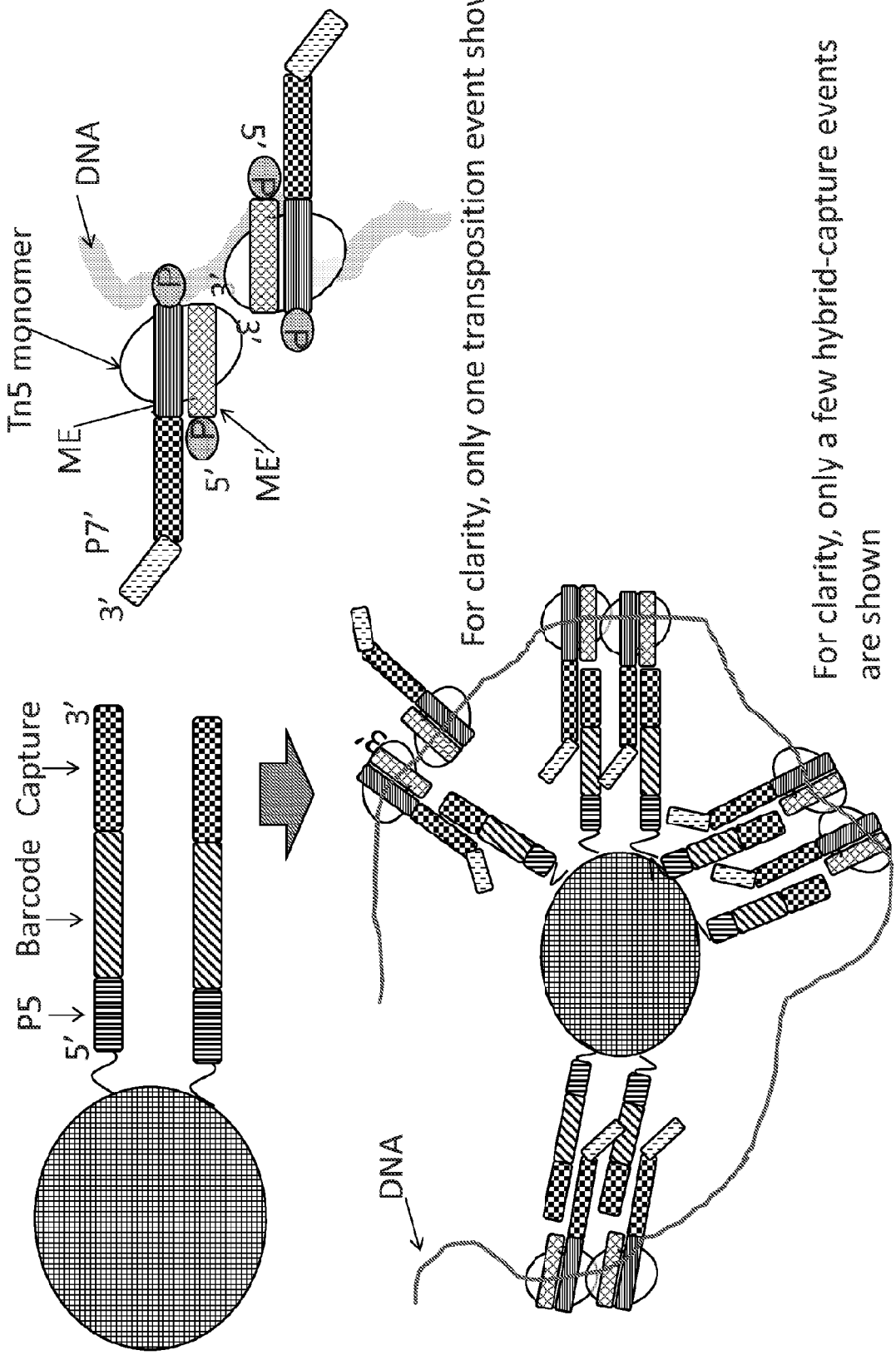
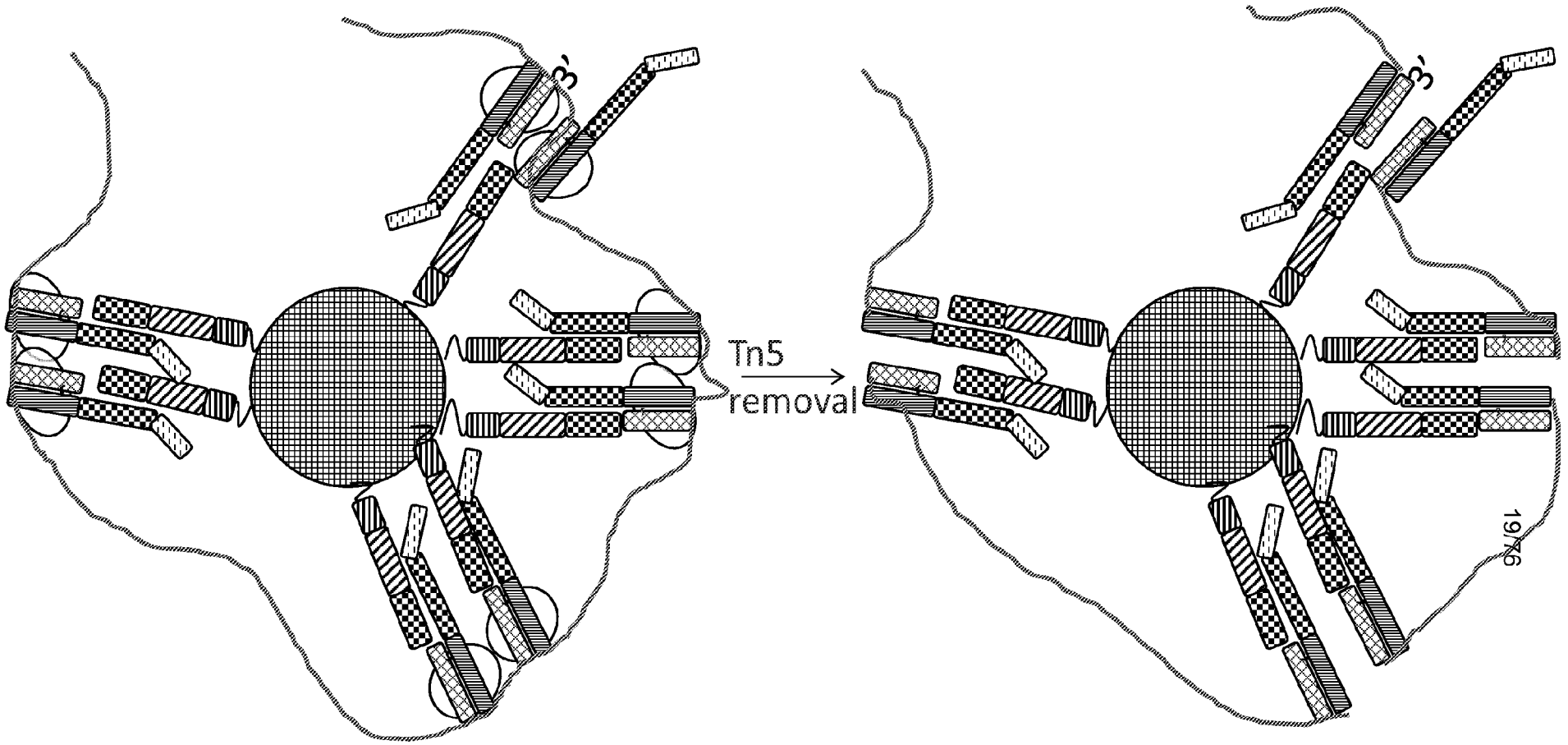

Tn5 monomer
DNA
ME
P7'
3'
5'
ME'
3'
5'
P5
Barcode
Capture
3'
5'
For clarity, only one transposition event shown
For clarity, only a few hybrid-capture events are shown
DNA

Figure 18



After hybridization capture, contiguity is maintained after Tn5 removal. DNA is hybridized to bead with indexed primers keeping all individual libraries together on a bead.

Figure 19

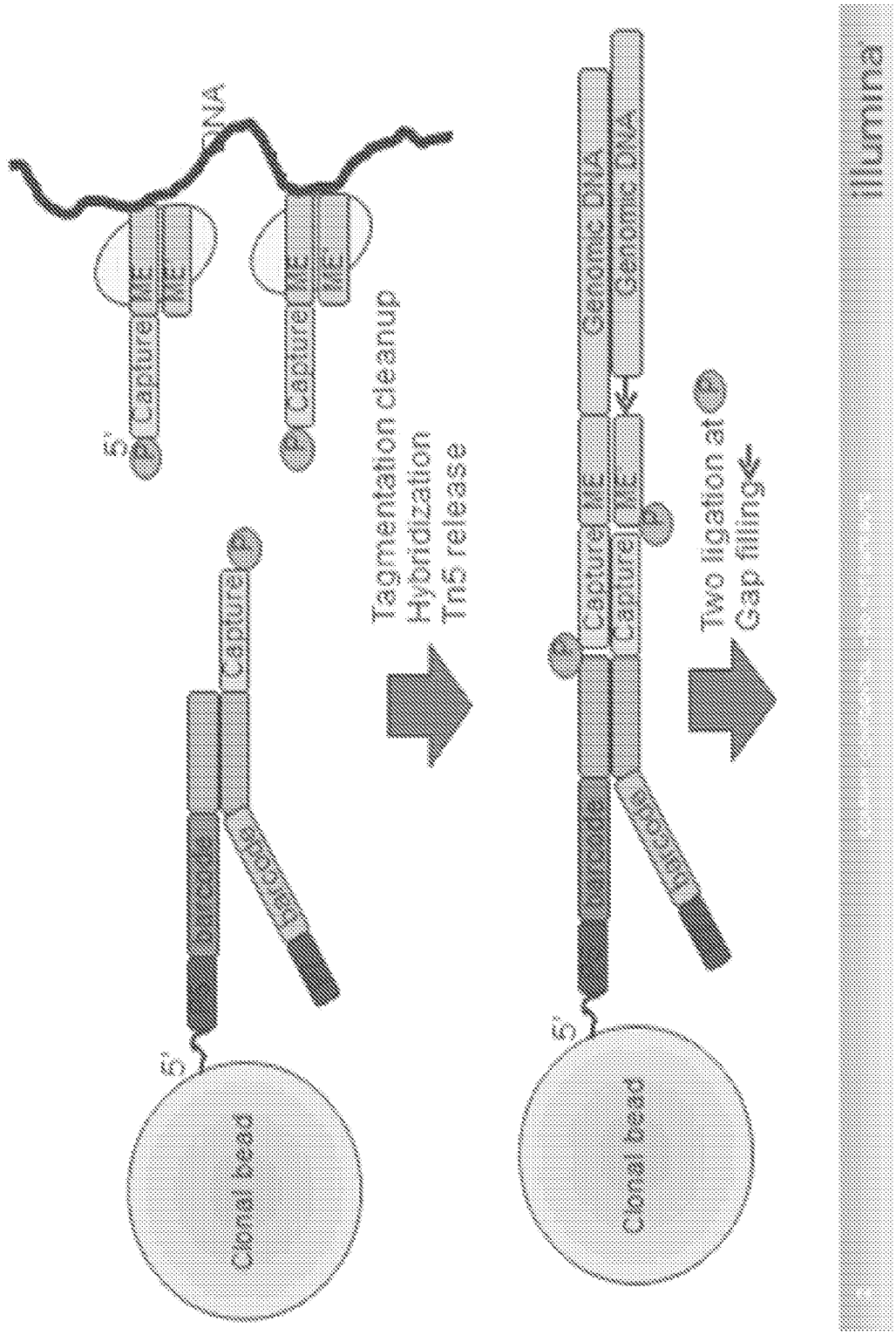

5'
Capture ME
ME
DNA
Capture ME
ME
Tagmentation cleanup
Hybridization
Tm5 release
Capture
Capture
Clonal bead
5'
Genomic DNA
Capture ME
ME
Capture ME
ME
Two ligation at
Gap filling
Clonal bead
5'
Illumina

Figure 20

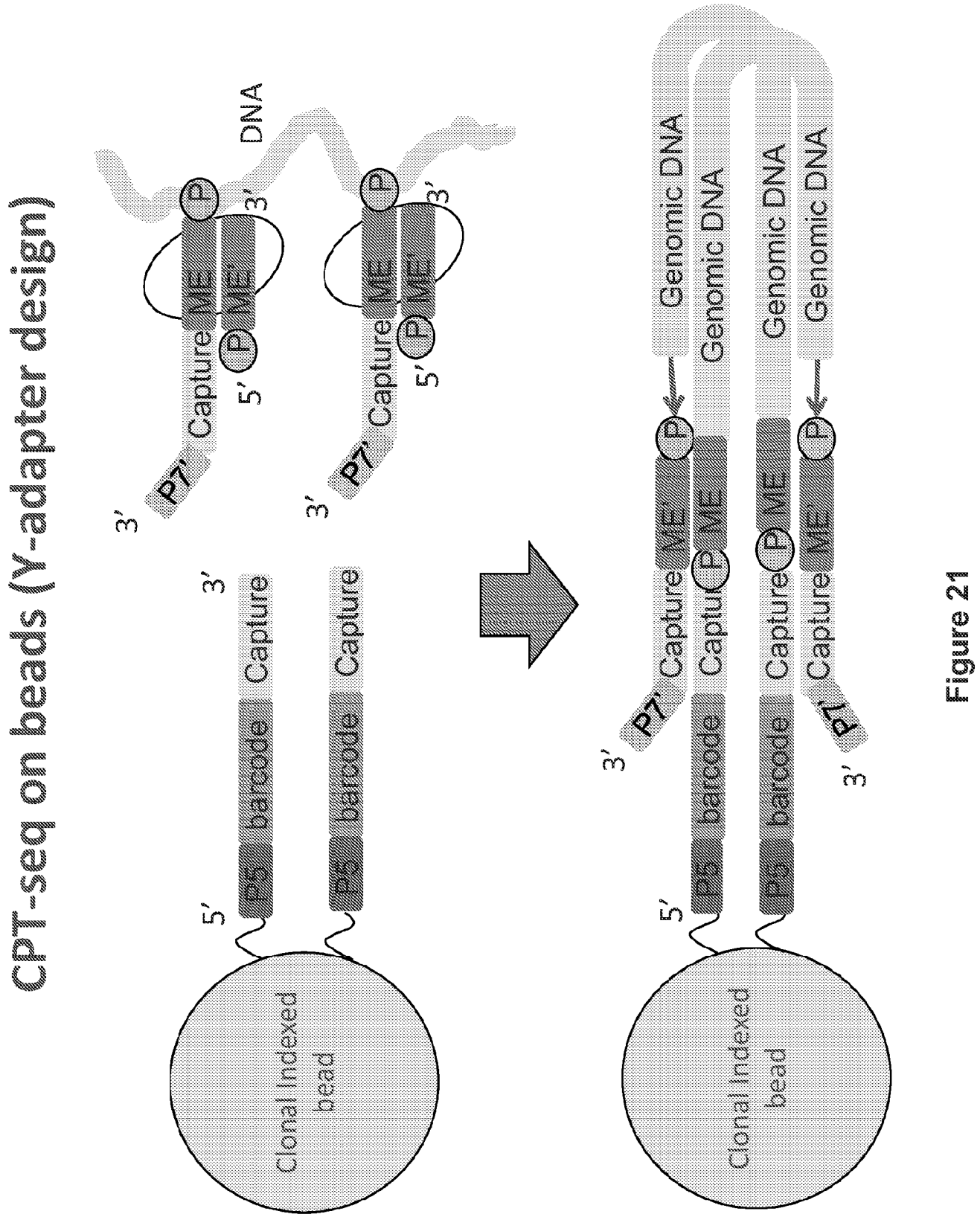

CPT-seq on beads (Y-adapter design)
DNA
3'
5'
P
ME
ME'
Capture
P7'
P5
barcode
Clonal Indexed bead
Genomic DNA
P1'

Figure 21

1. 100bp Ladder
2. gDNA
3. 12.5uM Tn5
4. 1uL NCP DNA
5. 1uL S-200 NCP DNA
6. 3uL S-200 NCP DNA
7. 6uL 2-200 NCP DNA
8. 1uL NCP DNA
9. 1uL S-400 NCP DNA
10. 3uL S-400 NCP DNA
11. 6uL S-400 NCP DNA
12. 1kb Ladder
Contiguously-linked Beads
Free transposome
3
illumina

Figure 22

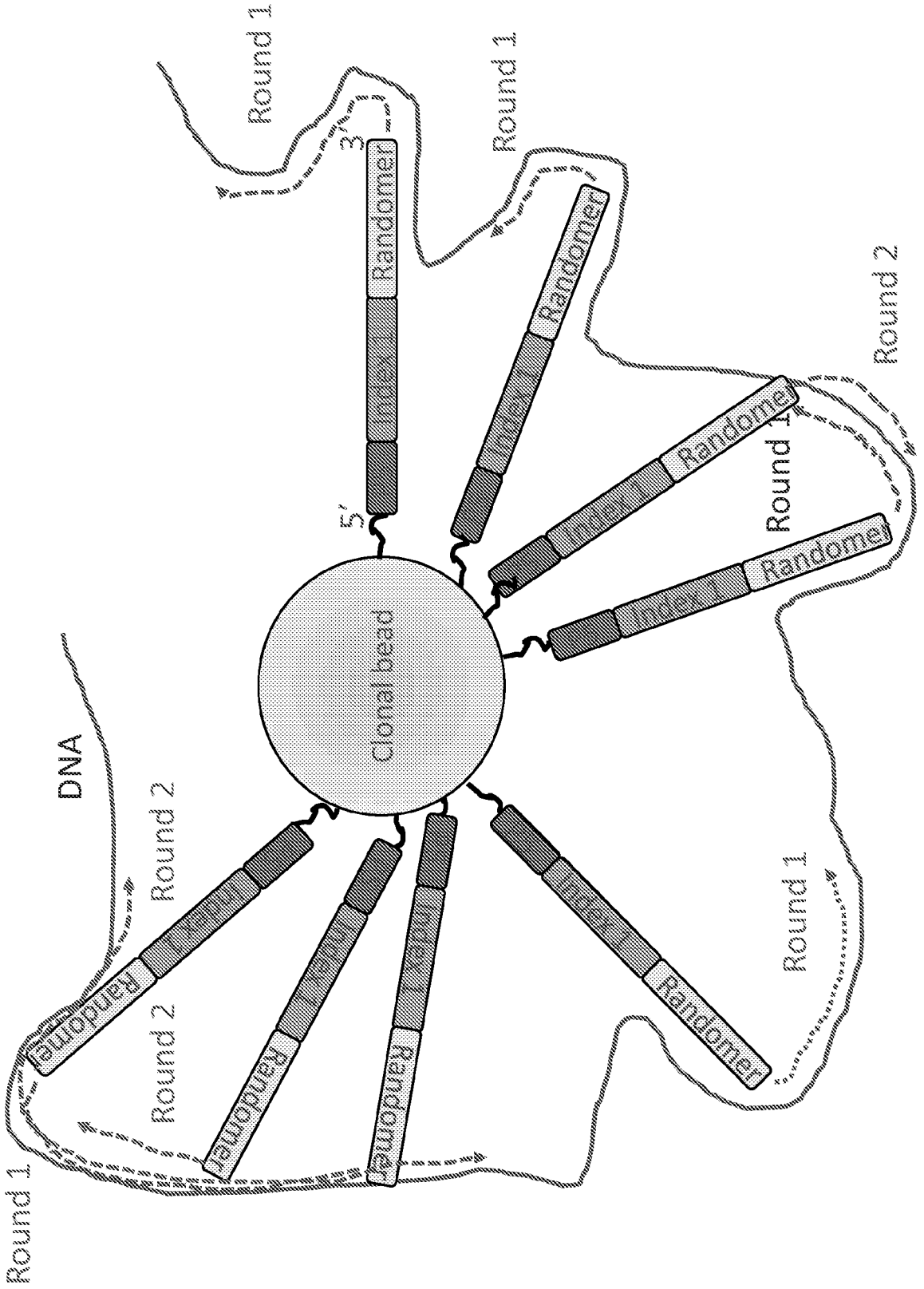
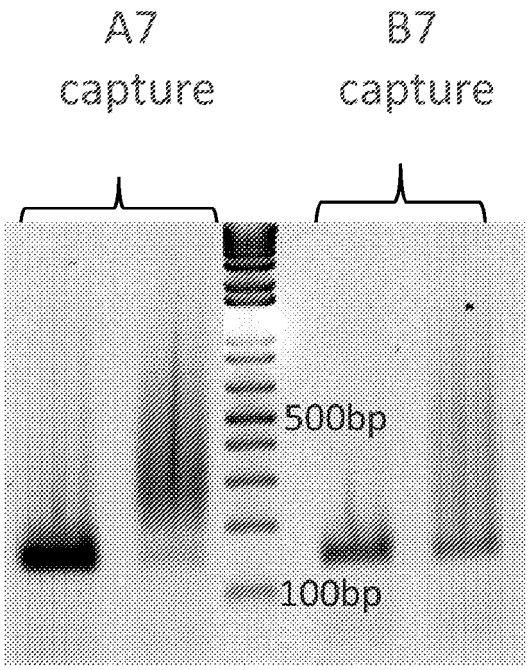

Round 1
Round 1
Round 2
Round 1
Round 2
Round 1
Round 1
Round 2
DNA
Clonal bead
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
Randomer
5'
3'

Figure 23

Indexed Beads w/ primers
(i) DNA
(ii) WGA
Sequencing library preparation
DNA fragment
Index 1
Index 1
Index 1
Index 1
Assembly
clonal indexed sequencing libraries

Figure 24

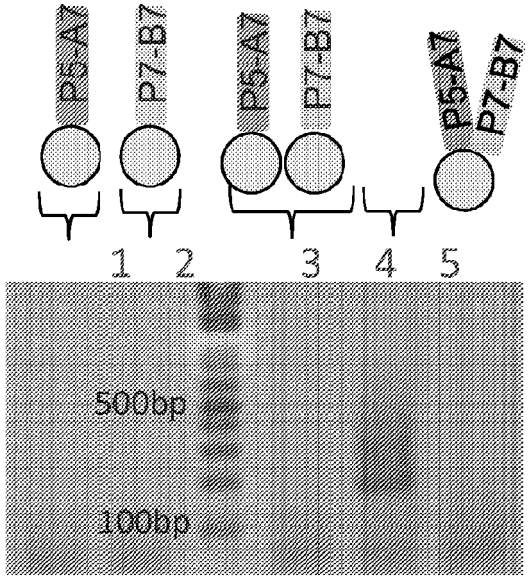
Optimization of Capture Probe Density on Beads



	Probe Density (dilution)	Ligation
1	1x	No
2	10x	Yes
3	1x	No
4	10x	Yes

Figure 25

Feasibility of CPT-seq on Beads: Intra-molecular Libraries



	Beads	Ligase Added
1	A7	Yes
2	B7	Yes
3	Bead A7 & B7	Yes
4	A7+B7	Yes
5	A7+B7	No

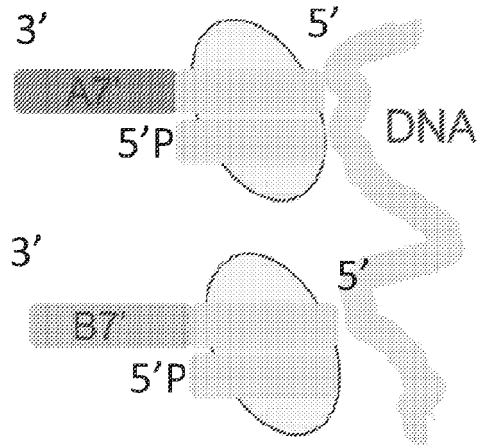
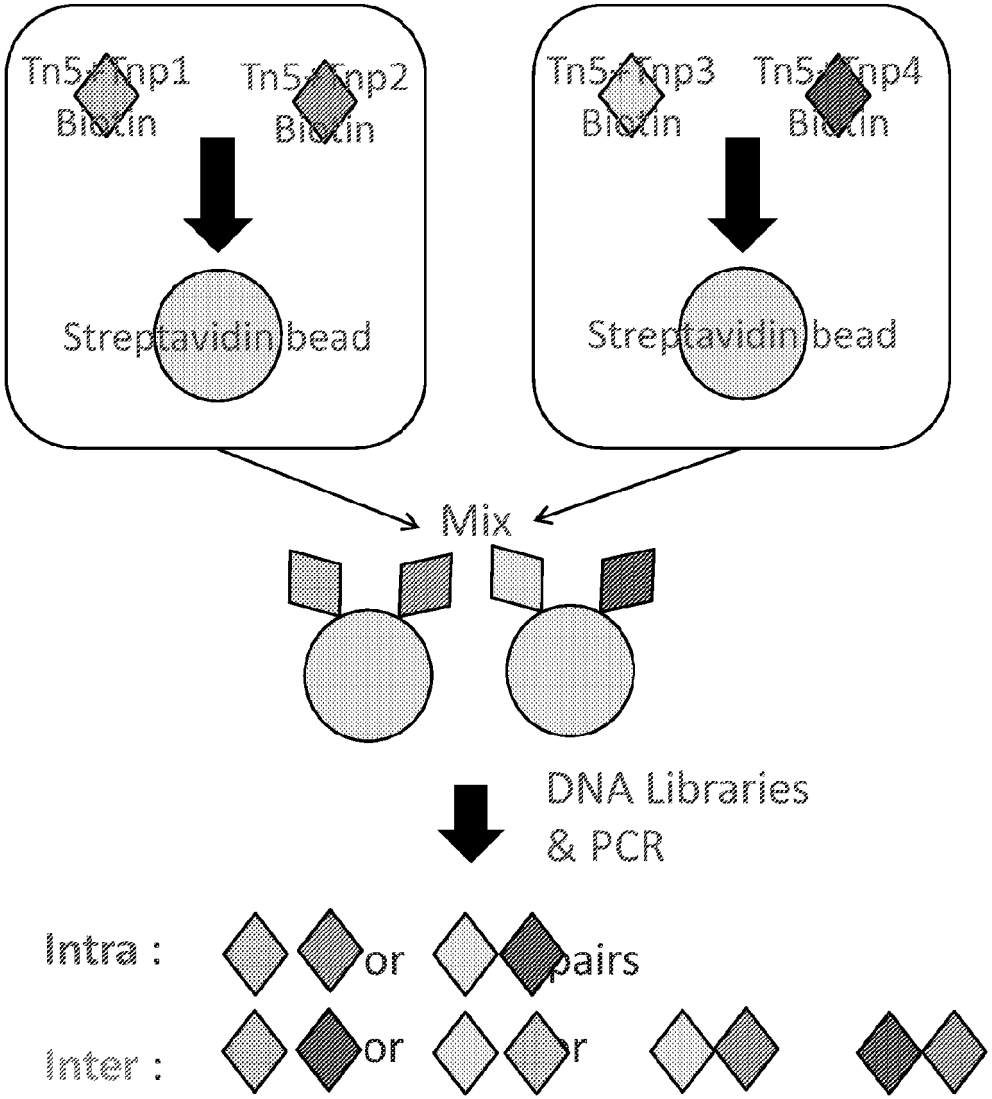
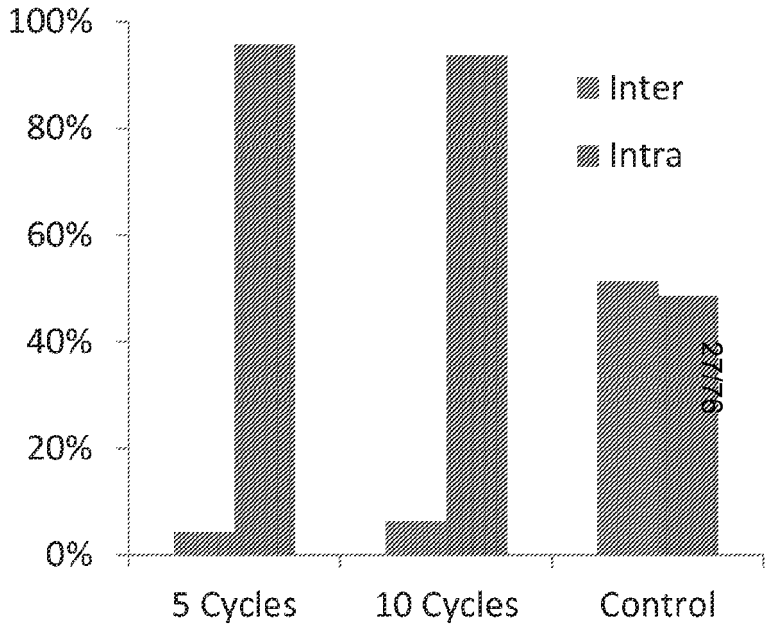


Figure 26

Feasibility of Clonal Indexing





- Clonal indexing observed

Figure 27

WO 2016/061517
PCT/US2015/056040

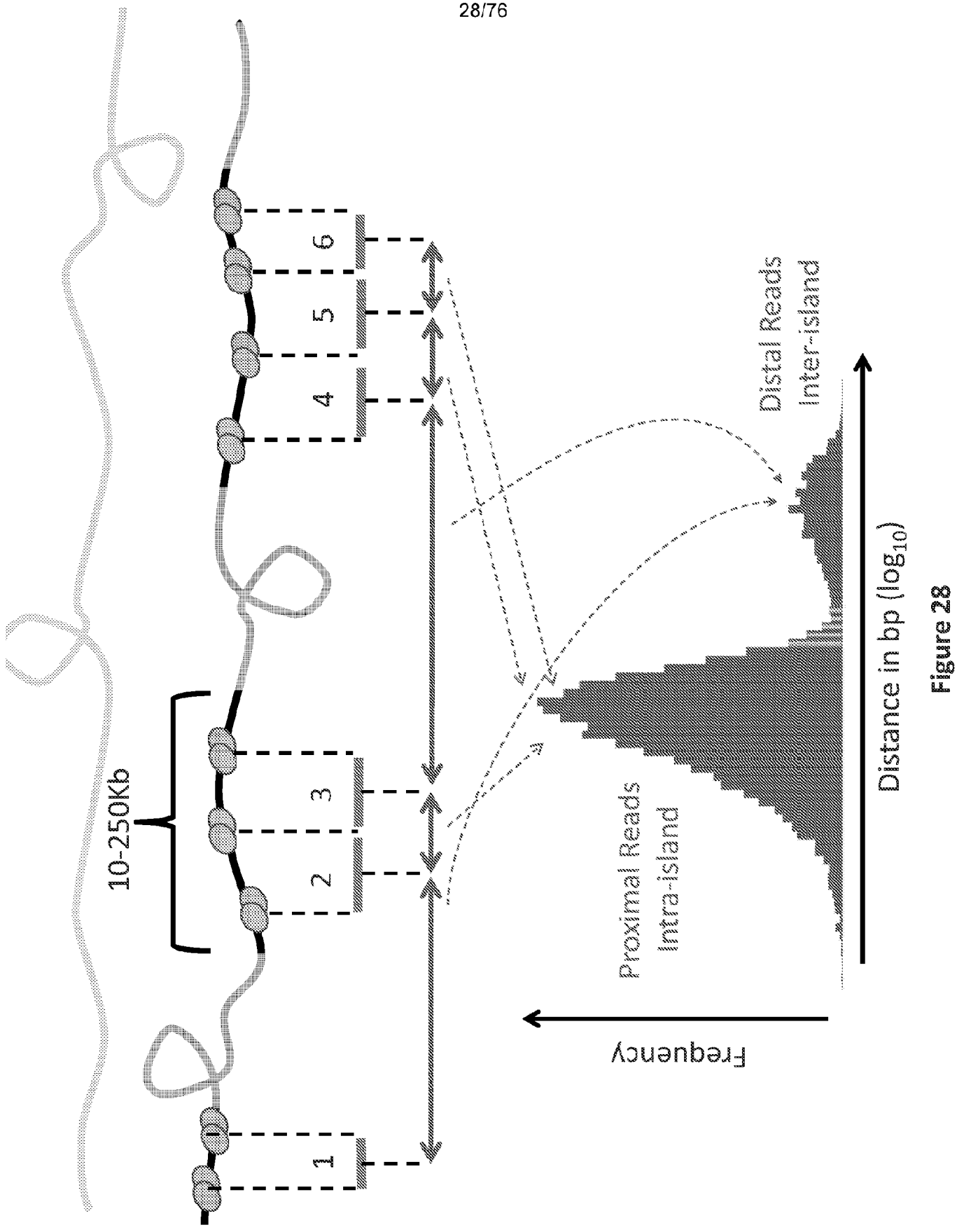
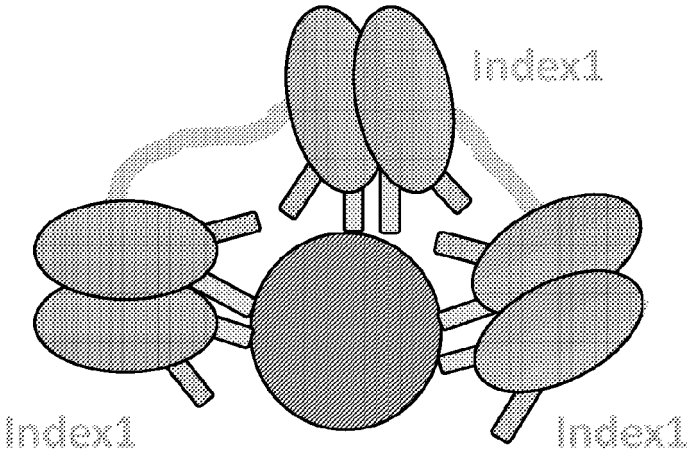

10-250Kb
1
2
3
4
5
6
Proximal Reads
Intra-island
Distal Reads
Inter-island
Frequency
Distance in bp (log10)

Figure 28

Approaches to Derive Contiguity Information on Solid Support

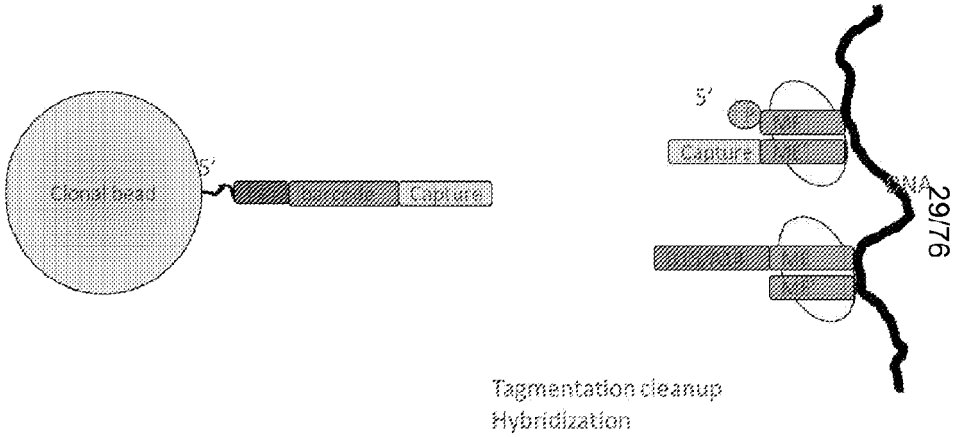
Clonal Indexed Transposomes on Beads



DNA (orange) is transposed by indexed transposomes on beads

FIG. 29A

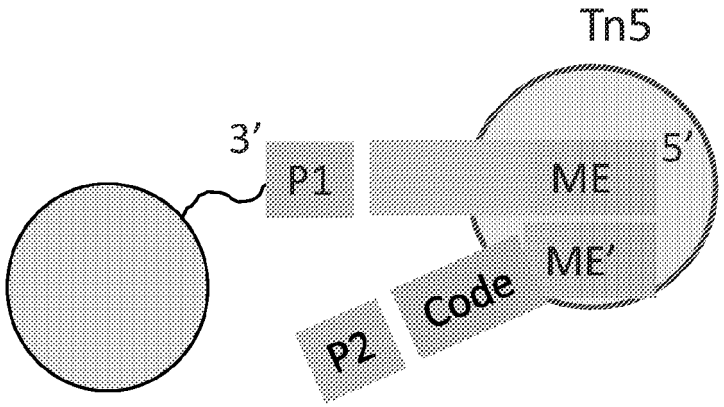
Clonal indexed capture probes on beads & CPT – DNA



Transposed DNA is captured by clonal indexed beads. Index is transferred to CPT- DNA

FIG.29B

One Pot 96 Indexed Clonal Bead Transposition



(only monomer shown for clarity)

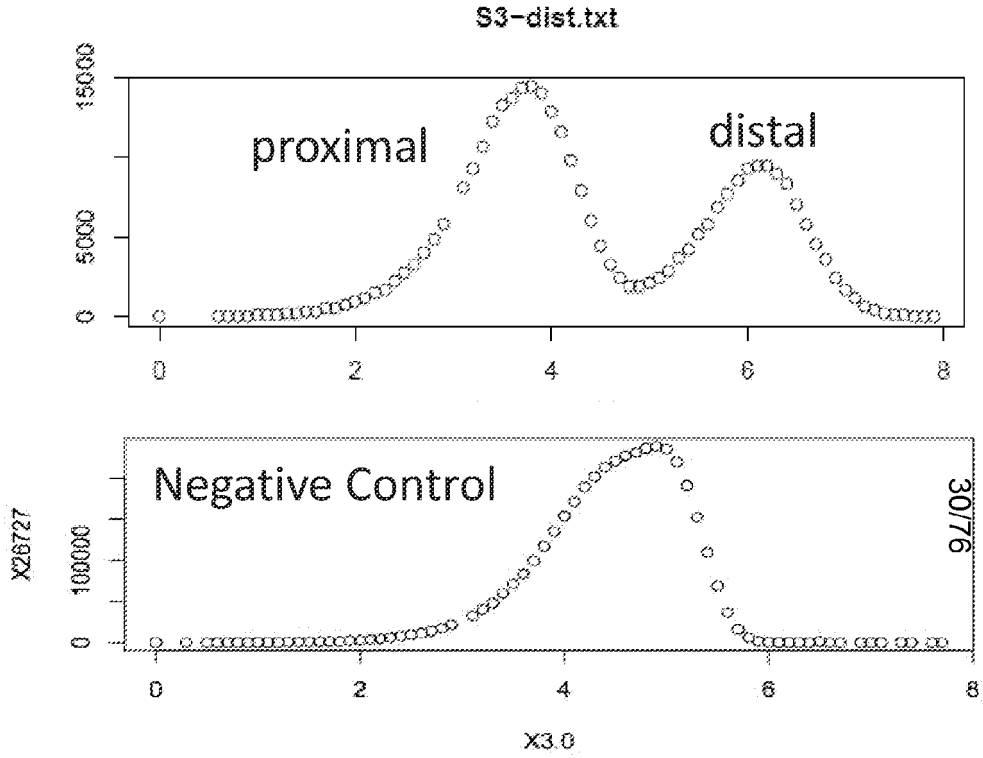
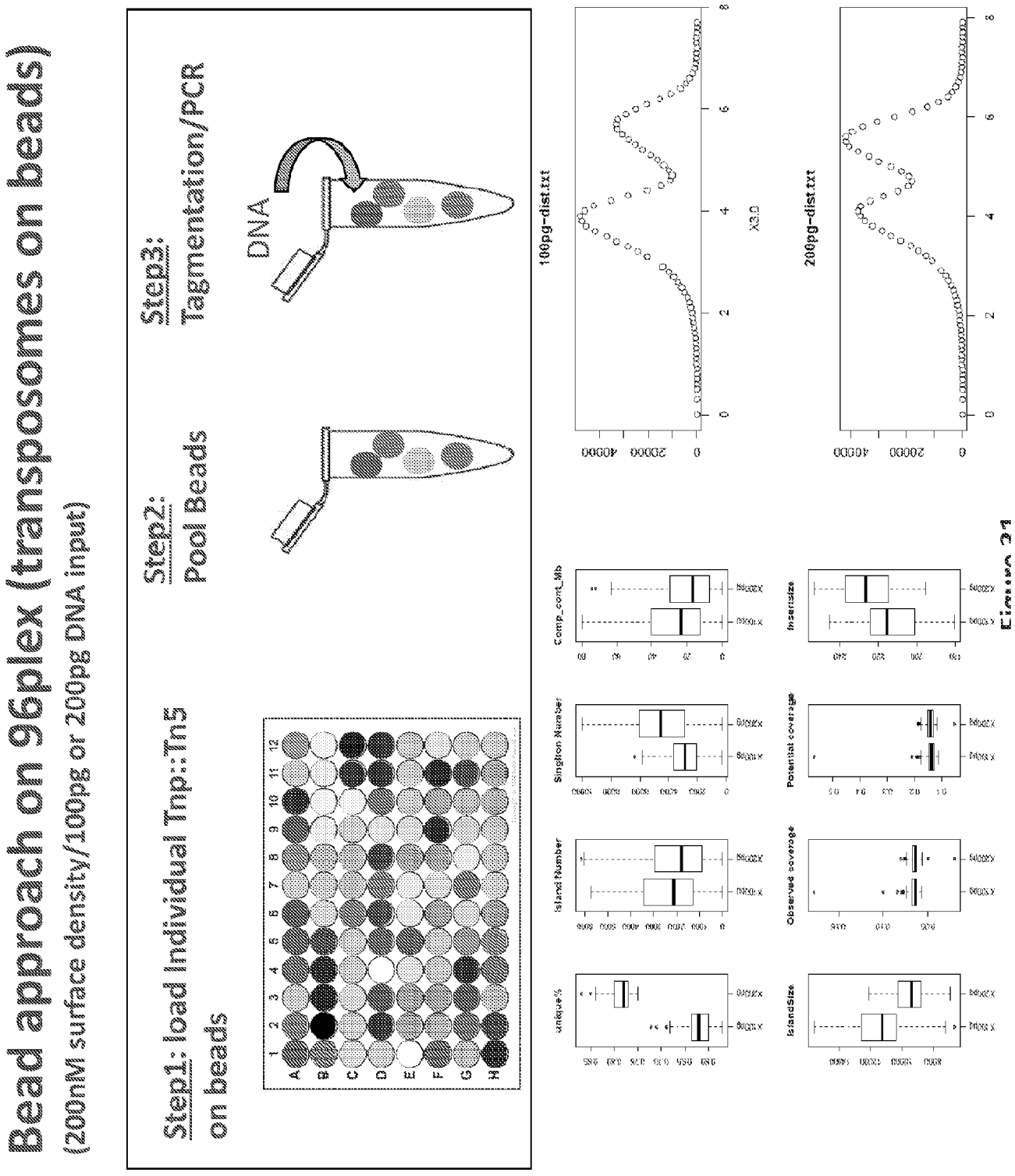
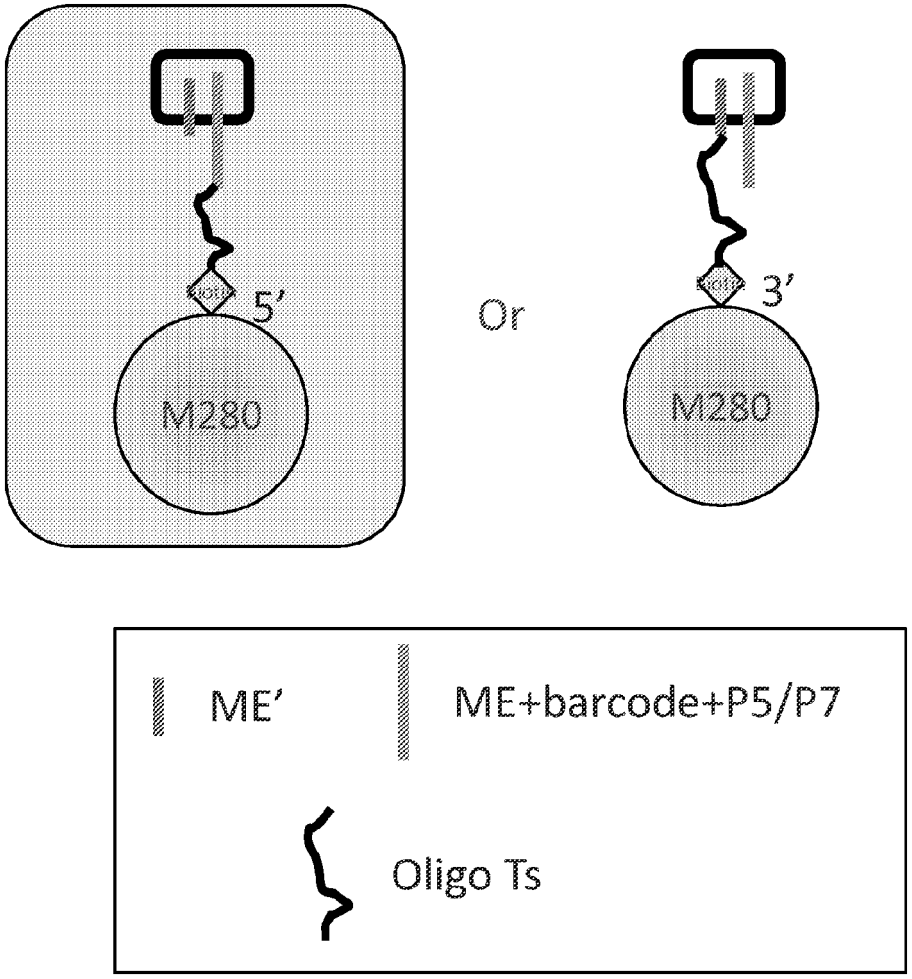


Figure 30


Bead approach on 96plex (transposomes on beads)
(200nM surface density/100pg or 200pg DNA input)
Step1: load Individual Tnp::Tn5 on beads
Step2: Pool Beads
Step3: Tagmentation/PCR
DNA
100pg-dist.txt
200pg-dist.txt
Comp_cont_Mb
Singlton Number
Island Number
Unique%
Insertsize
Potential coverage
Observed coverage
IslandSize
Extended Data Fig. 3f

5’biotin ME and 3’biotin ME’ oligos on beads



- Assembly Tnp:Tn5 complex first
- Load on beads
- Wash beads
- Mix beads
- Add DNA and tagmentation
- PCR

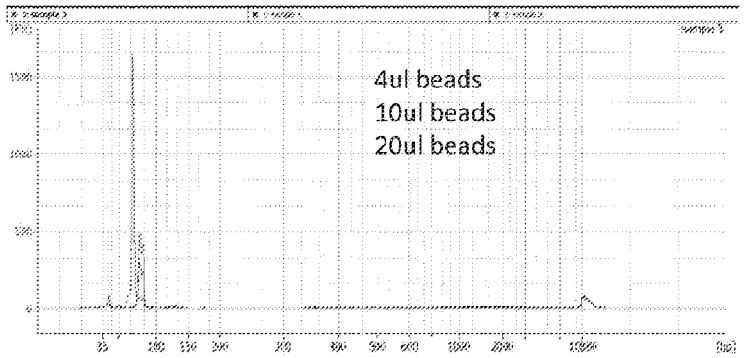
Figure 32

Library Sizes for Transposomes on Beads

No SDS, 5 cycles PCR

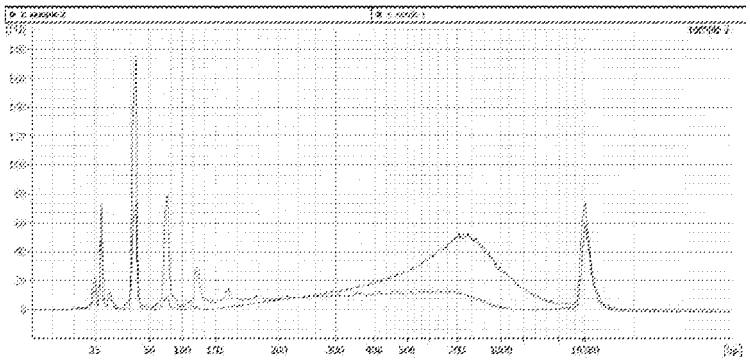
SDS release before 5 cycles PCR

5’ biotin

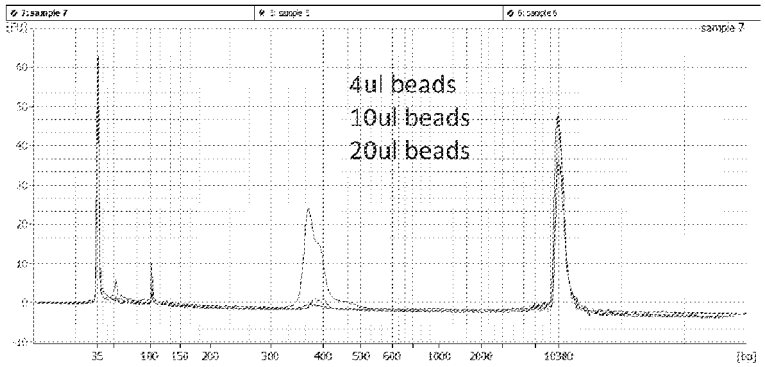


Read trace is control without beads

5’ biotin



3’ biotin



3’ biotin

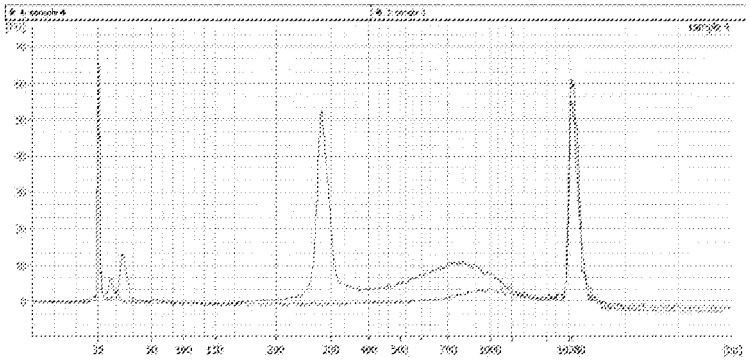
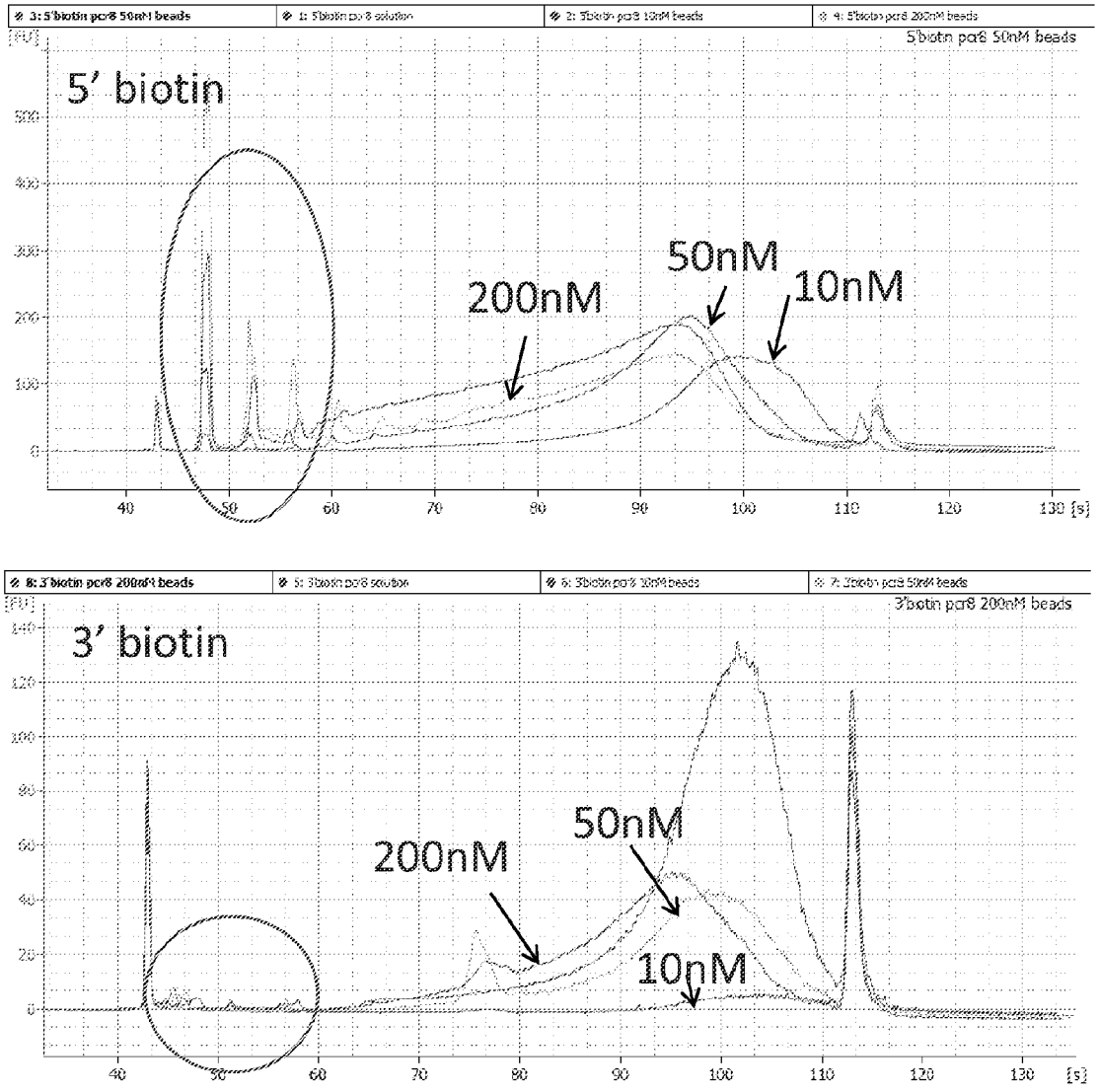


Figure 33

Transposome Surface density vs insertion size

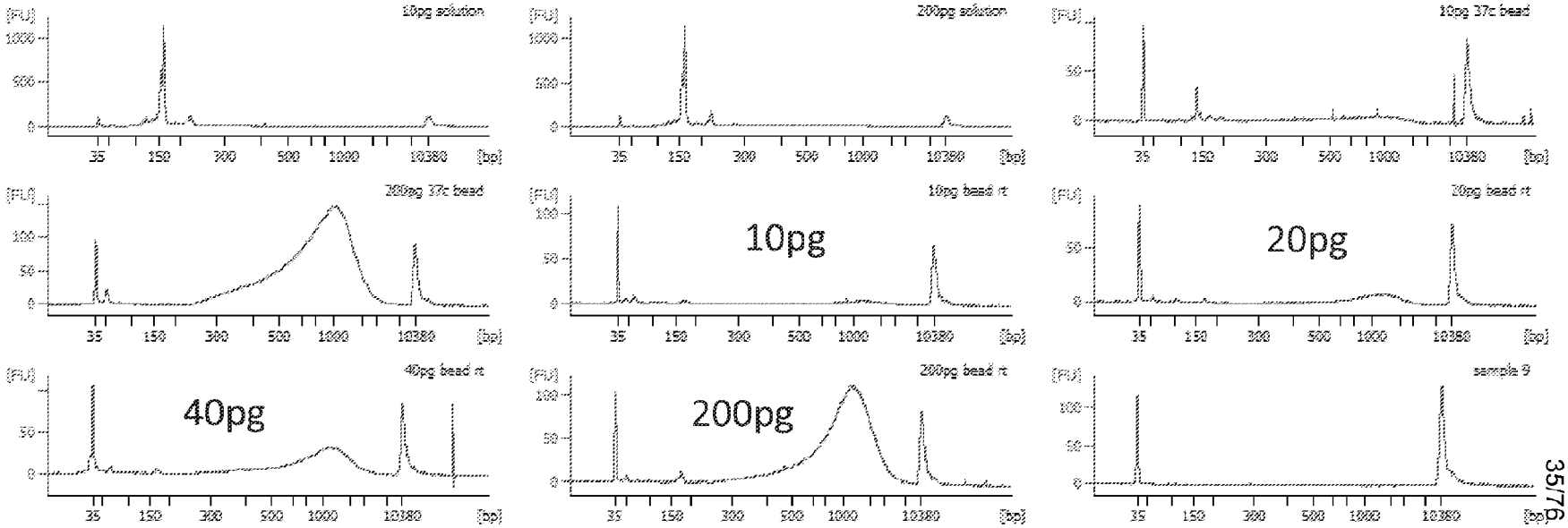


- 10ng input DNA
- Tagmentation @RT
- 8 cycles PCR
- Solution 25nM Tnp:Tn5
- EDTA added after tagmentation
- Wash beads 2x
- 0.1% SDS release before PCR

- 5'biotin has higher effieciency
- 5'biotin shows more self insertion by-products
- 5'biotin shows smaller sized library

Figure 34

DNA input titration



- ✓ 10pg input might be too low
- ✓ Size distribution is independent on the input DNA amount
- ✓ 37c doesn't change the size distribution

Figure 35

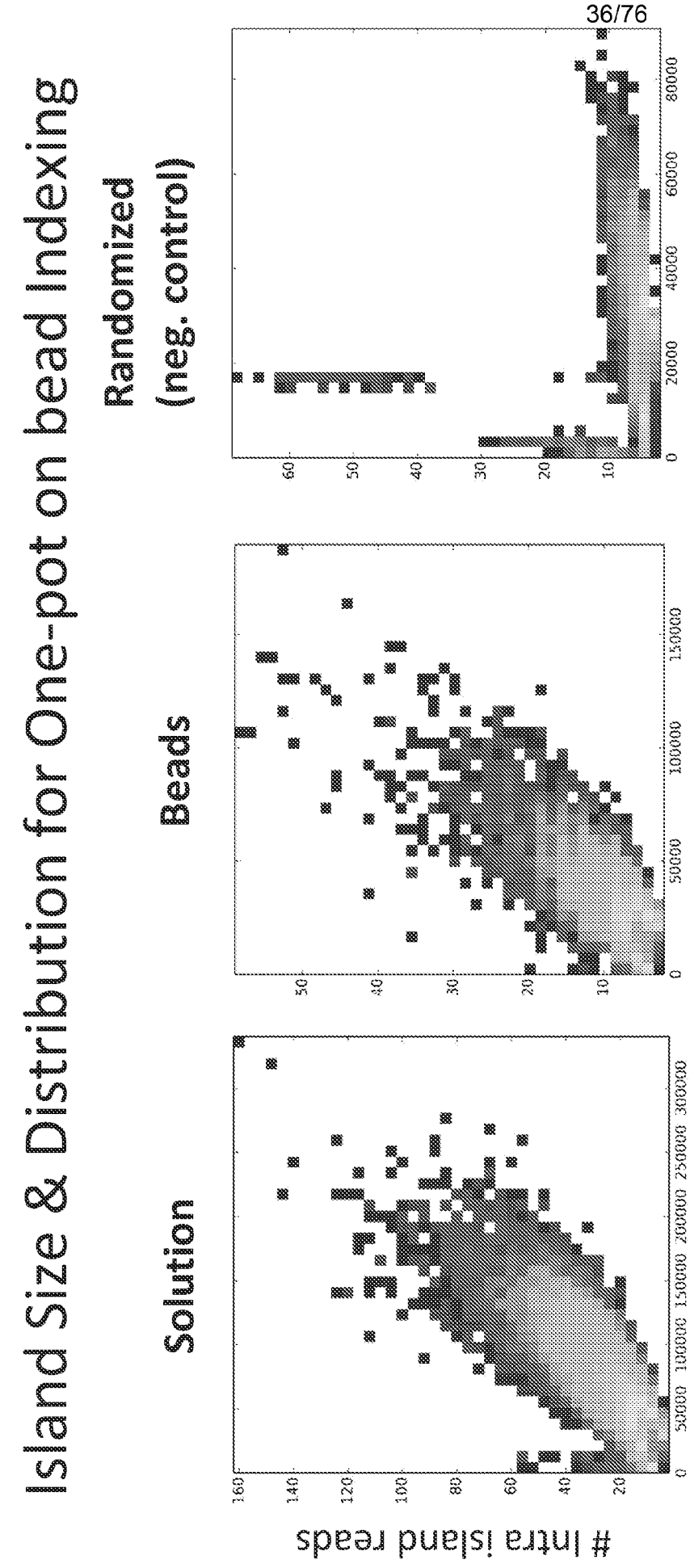

Island Size & Distribution for One-pot on bead Indexing
Randomized
(neg. control)
Beads
Solution
Intra island reads
Island Size

Figure 36

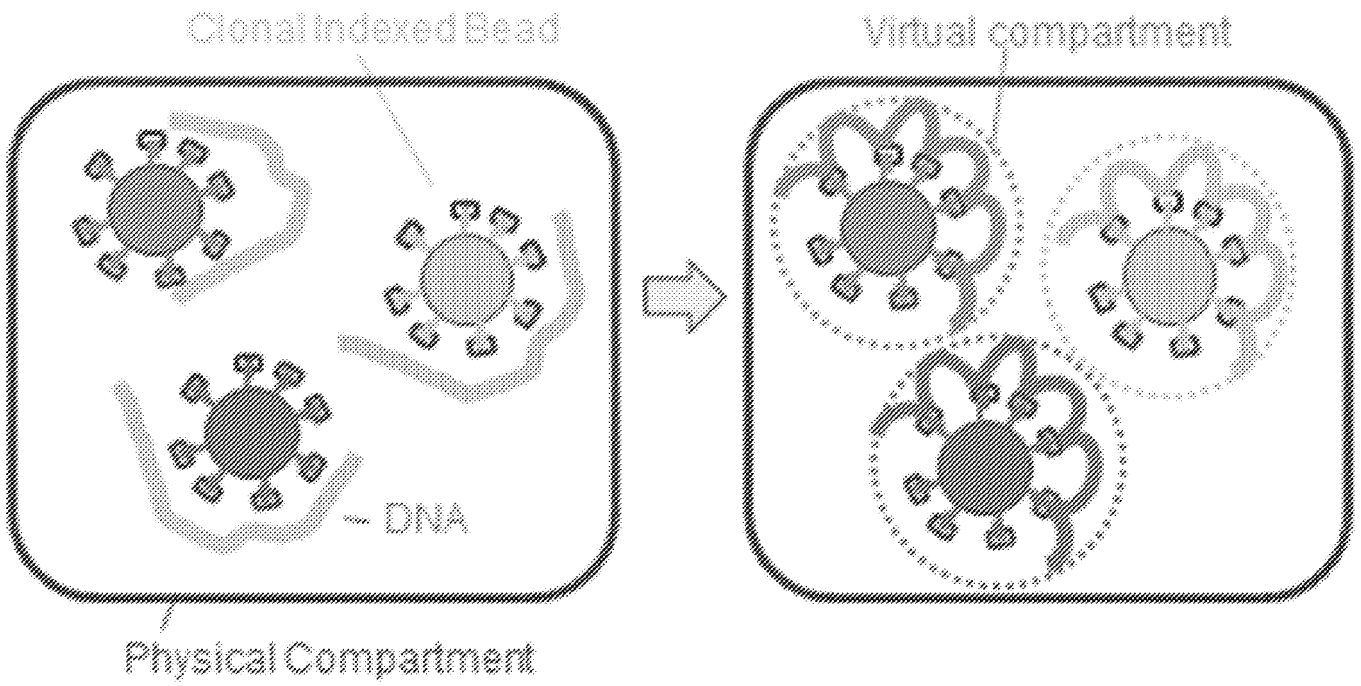

Clonal Indexing Across DNA Molecules
Clonal Indexed Bead
Virtual compartment
~ DNA
Physical Compartment
DNA in proximity of clonal bead receives same index
Intra >>> inter molecular indexing
5 um bead sufficient to capture 100kb contiguity (C=15 um, 100kb ~34um)
illumina

Figure 37

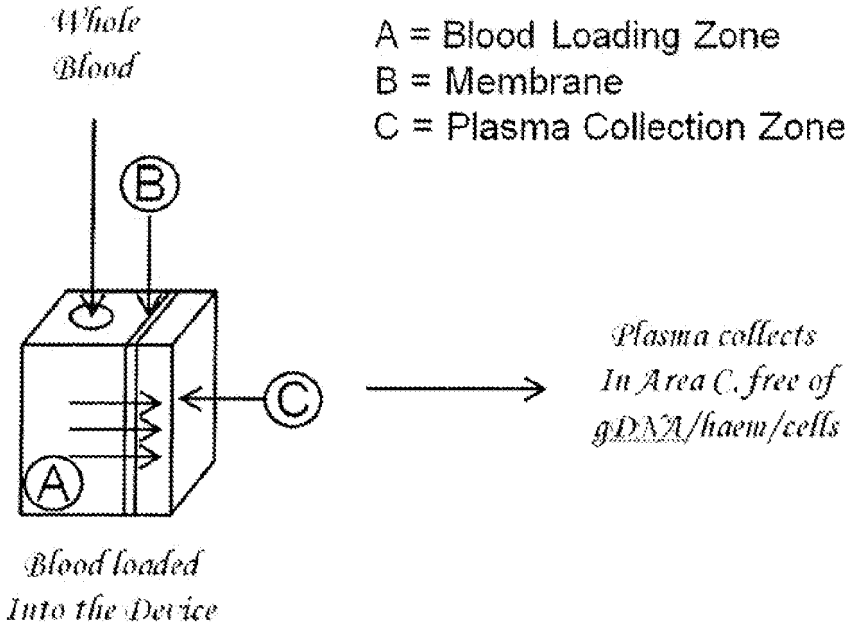

Whole Blood
A = Blood Loading Zone
B = Membrane
C = Plasma Collection Zone
B
C
A
Plasma collects In Area C free of gDNA/haem/cells
Blood loaded Into the Device

Figure 38

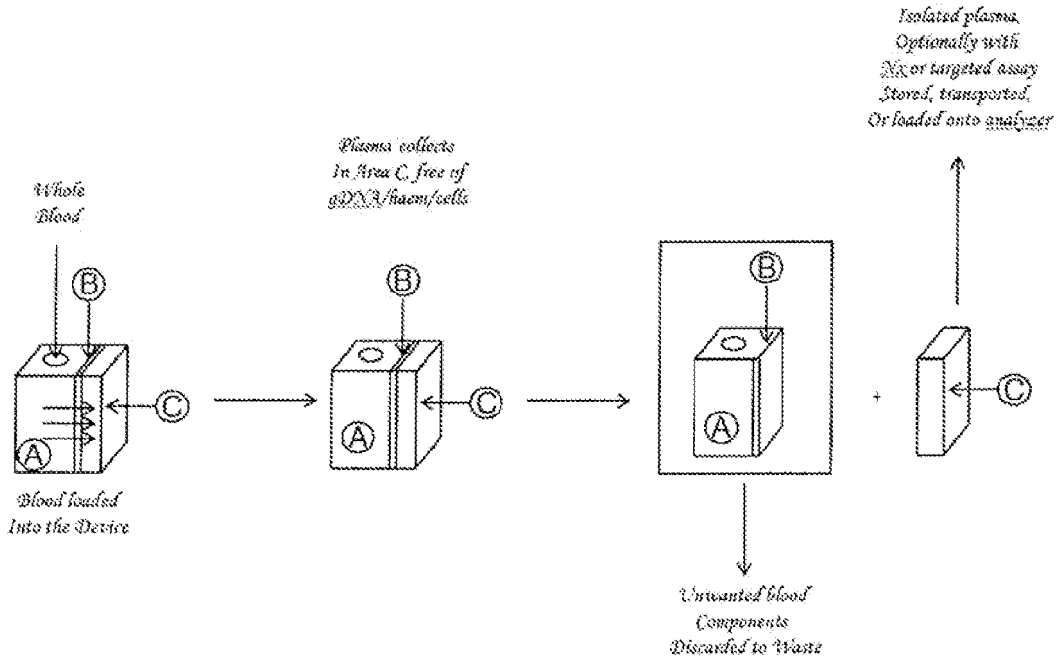

Whole Blood
Plasma collects In Area C free of gDNA/haem/cells
Isolated plasma, Optionally with NK or targeted assay Stored, transported, Or loaded onto analyzer
Blood loaded Into the Device
Unwanted blood Components Discarded to Waste
A
B
C

Figure 39

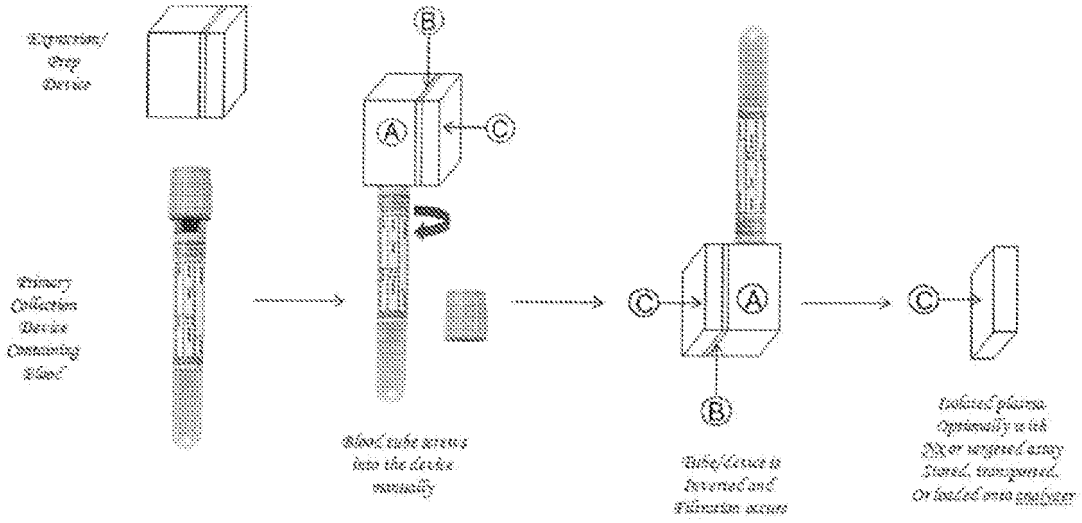

Expressway Prep Device
Primary Collection Device Containing Blood
Blood tube screws into the device manually
Tube/device is inverted and Filtration occurs
Isolated plasma Optionally with DNA or targeted assay Stored, transported, Or loaded onto analyzer
A
B
C

Figure 40

CPT-Seq Enrichment

- Targeted enrichment of whole-genome indexed-linked libraries
- Sample indexing + enrichment compatible

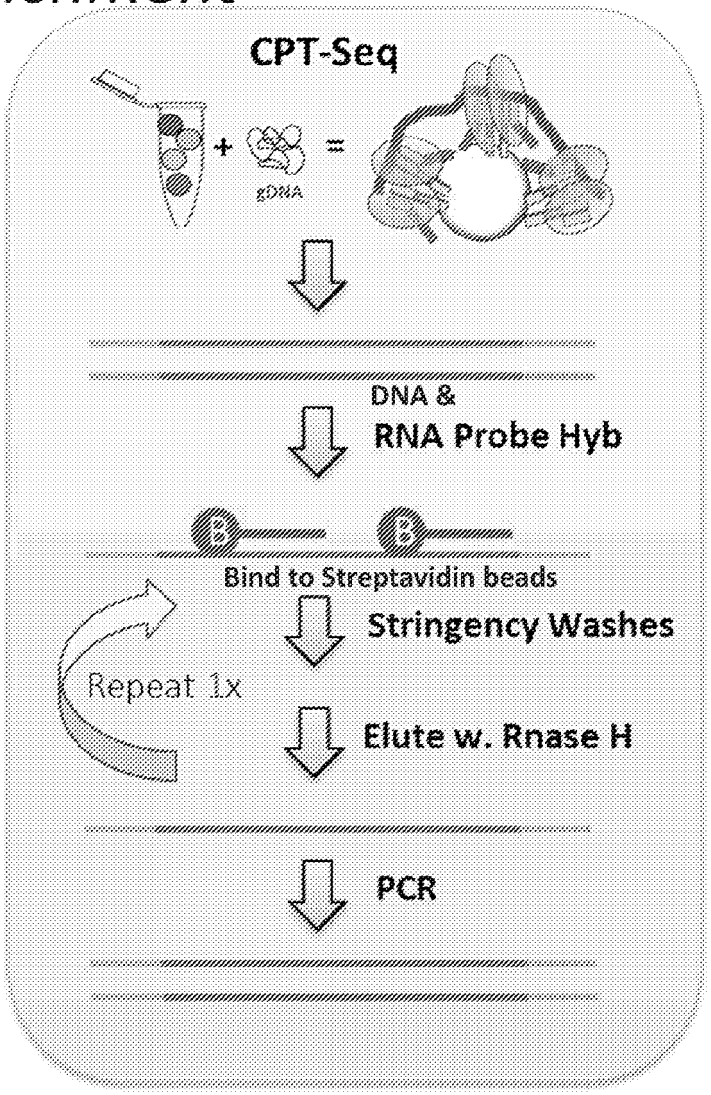


Fig. 41

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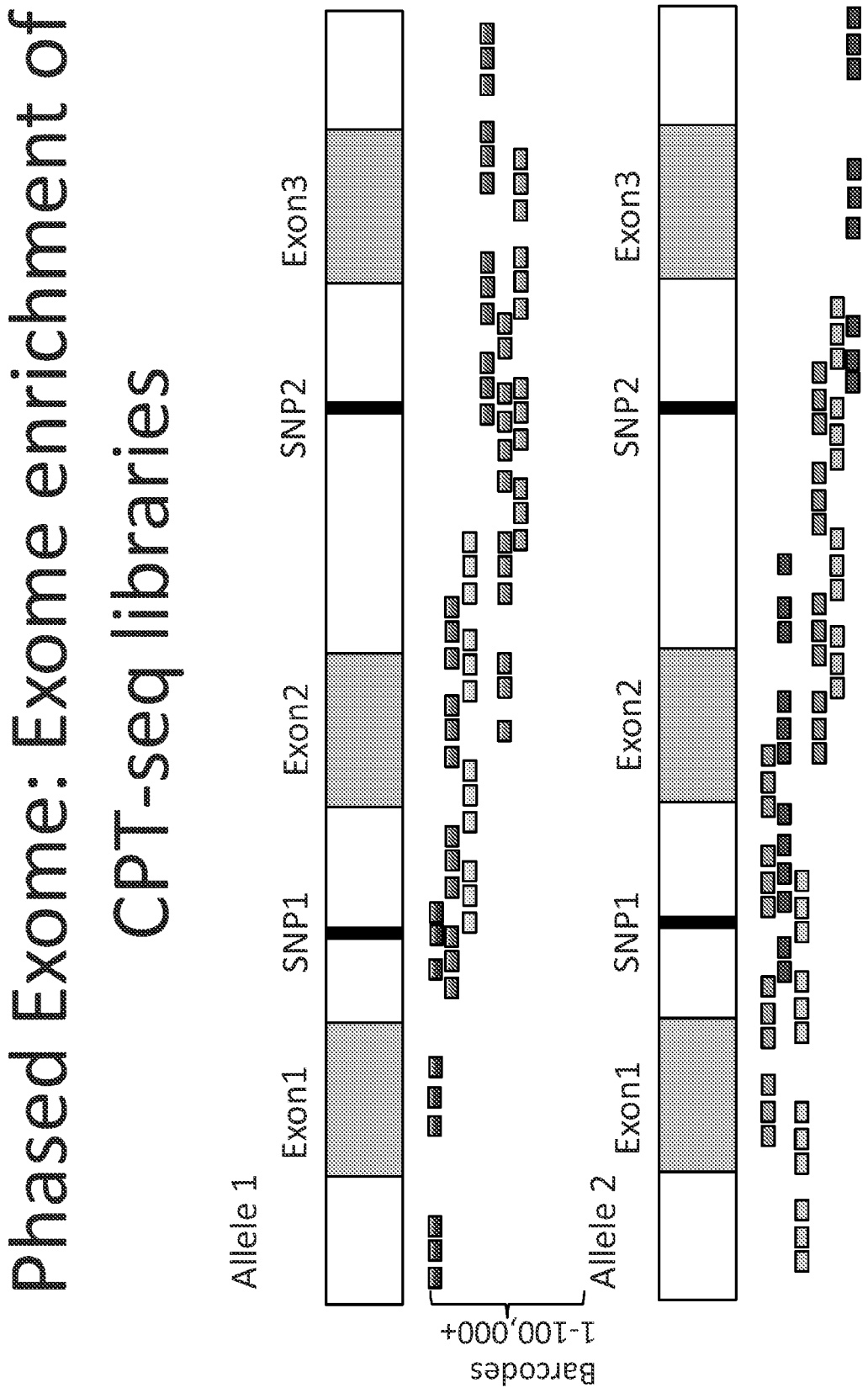

Phased Exome: Exome enrichment of CPT-seq libraries
Allele 1
Exon1
SNP1
Exon2
SNP2
Exon3
Allele 2
Exon1
SNP1
Exon2
SNP2
Exon3
1-100,000+
Barcodes

Fig. 42

Methyl CPT-Seq Workflow 1

gDNA Tagmented on beads

CPT-seq library on beads

BS converted CPT-seq library on beads

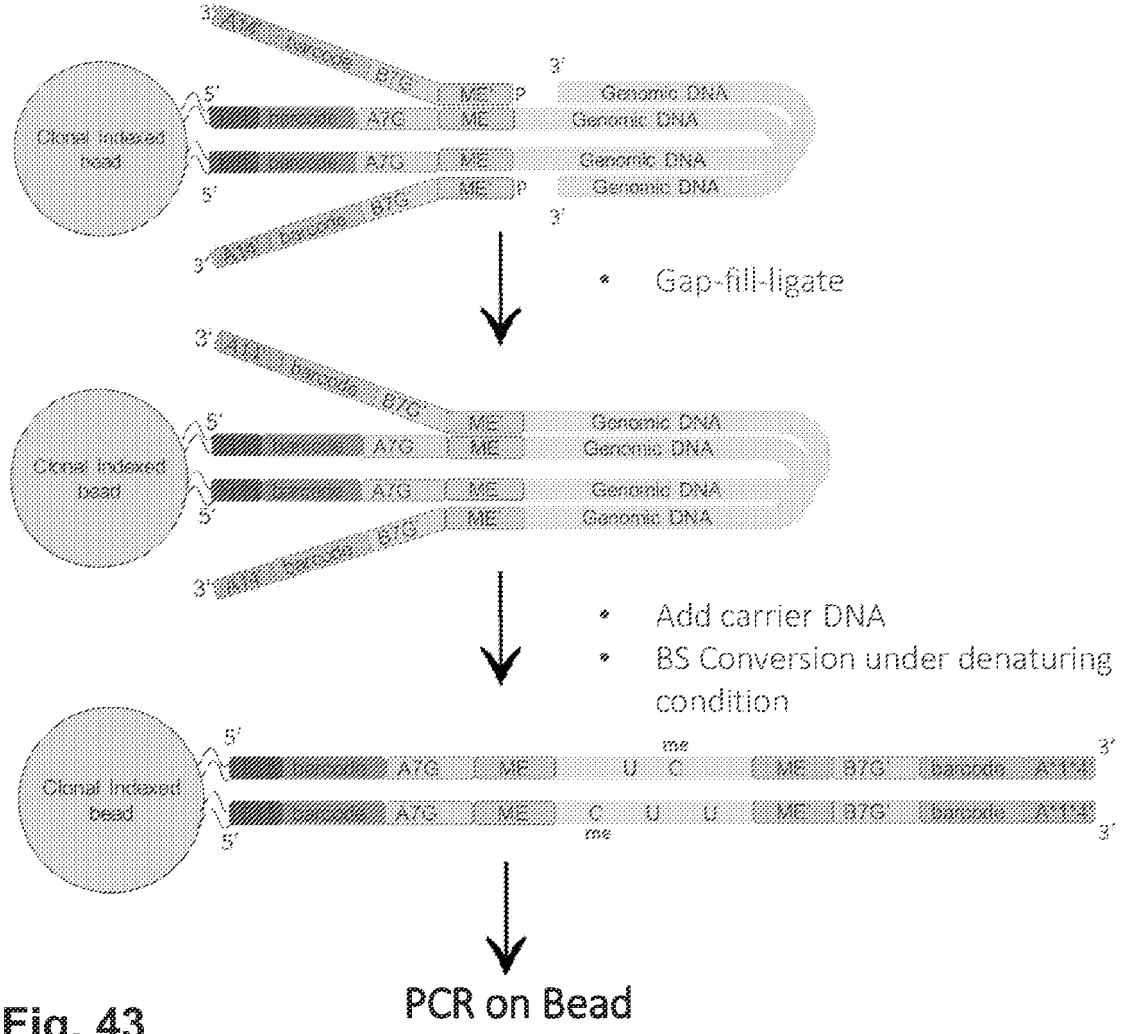
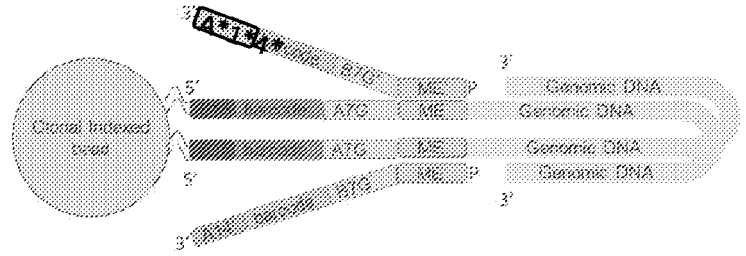


Fig. 43

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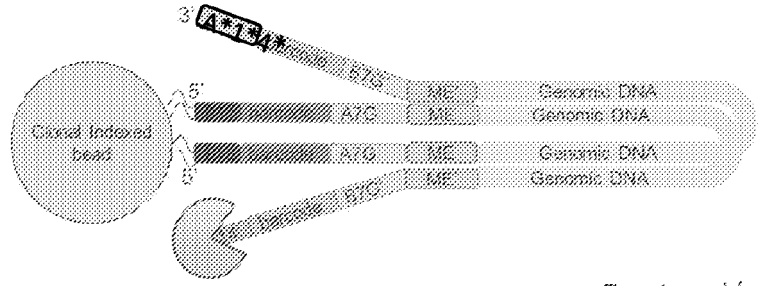
Example Methyl CPT-Seq Workflow 2

Genomic DNA tagmented on beads. 50% of 3′ ends contain thio-phophate backbone.



- Gap-fill-ligate

CPT-seq library on beads



- Exo I and/or Exo III Digestion
- Add carrier DNA
- BS Conversion

BS converted CPT-seq library on beads

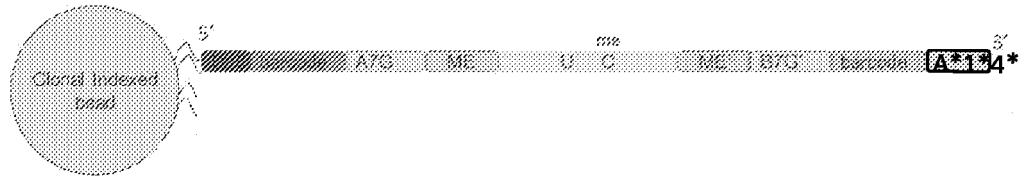


Fig. 44

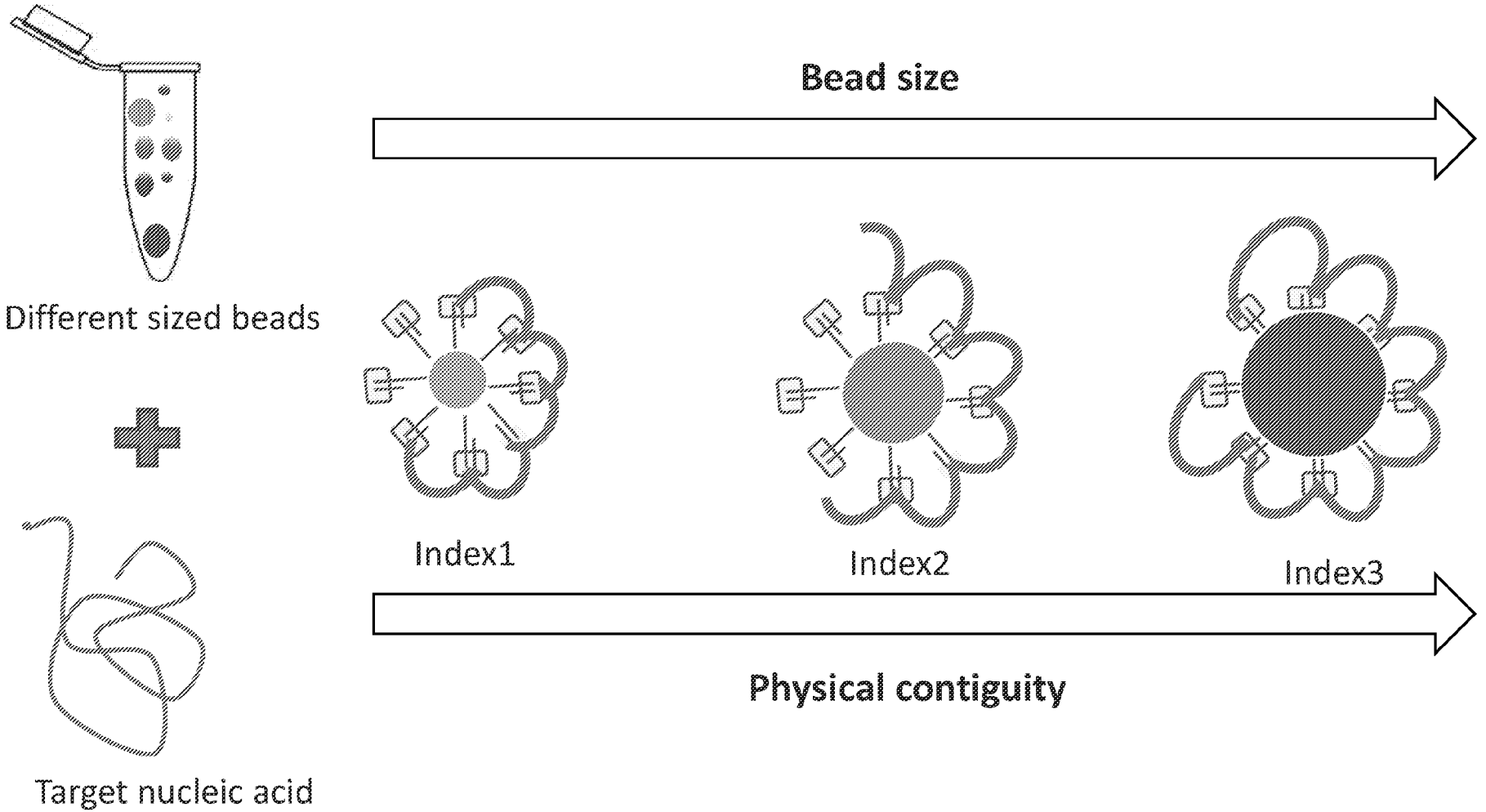


Fig. 45

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Example of assembly/SV characterization w/ different length scale libraries

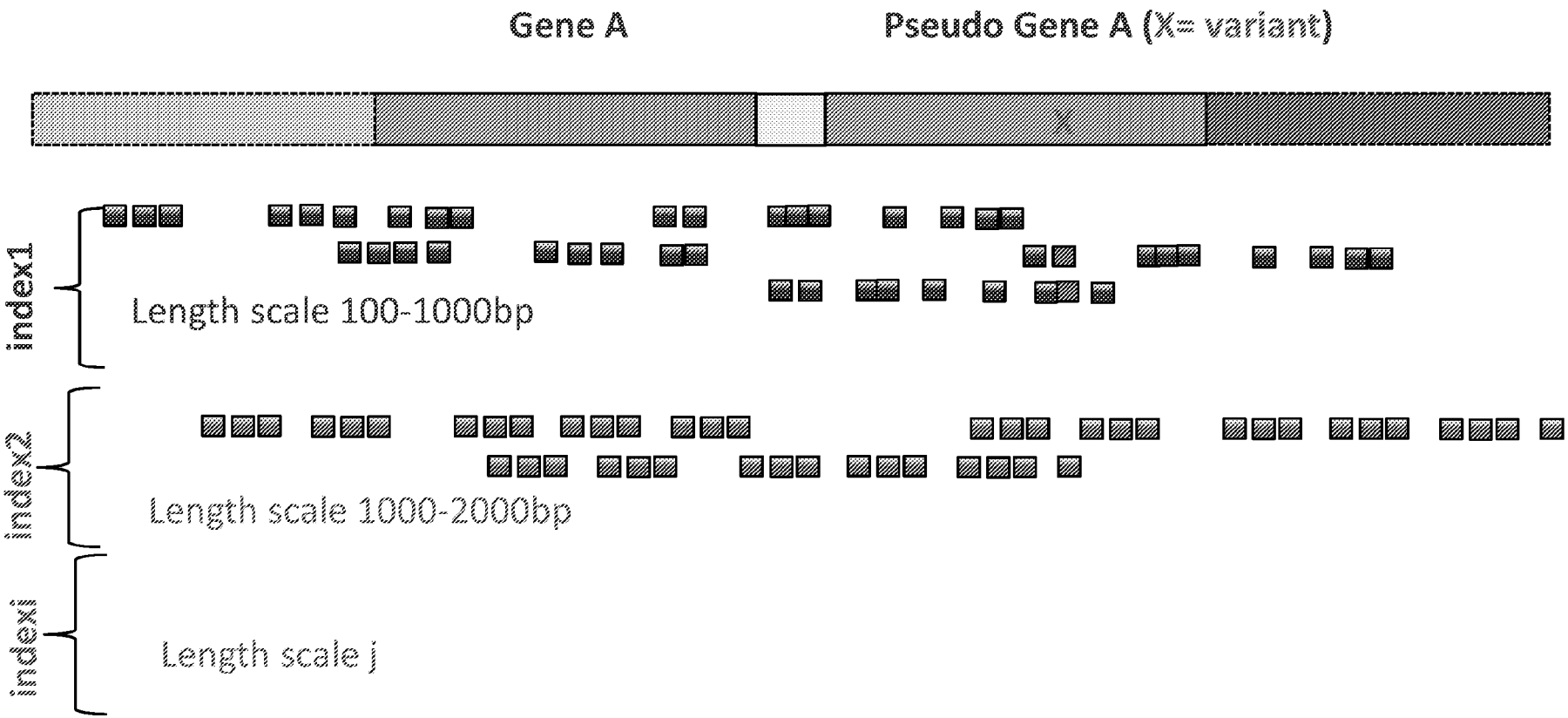


Fig. 46

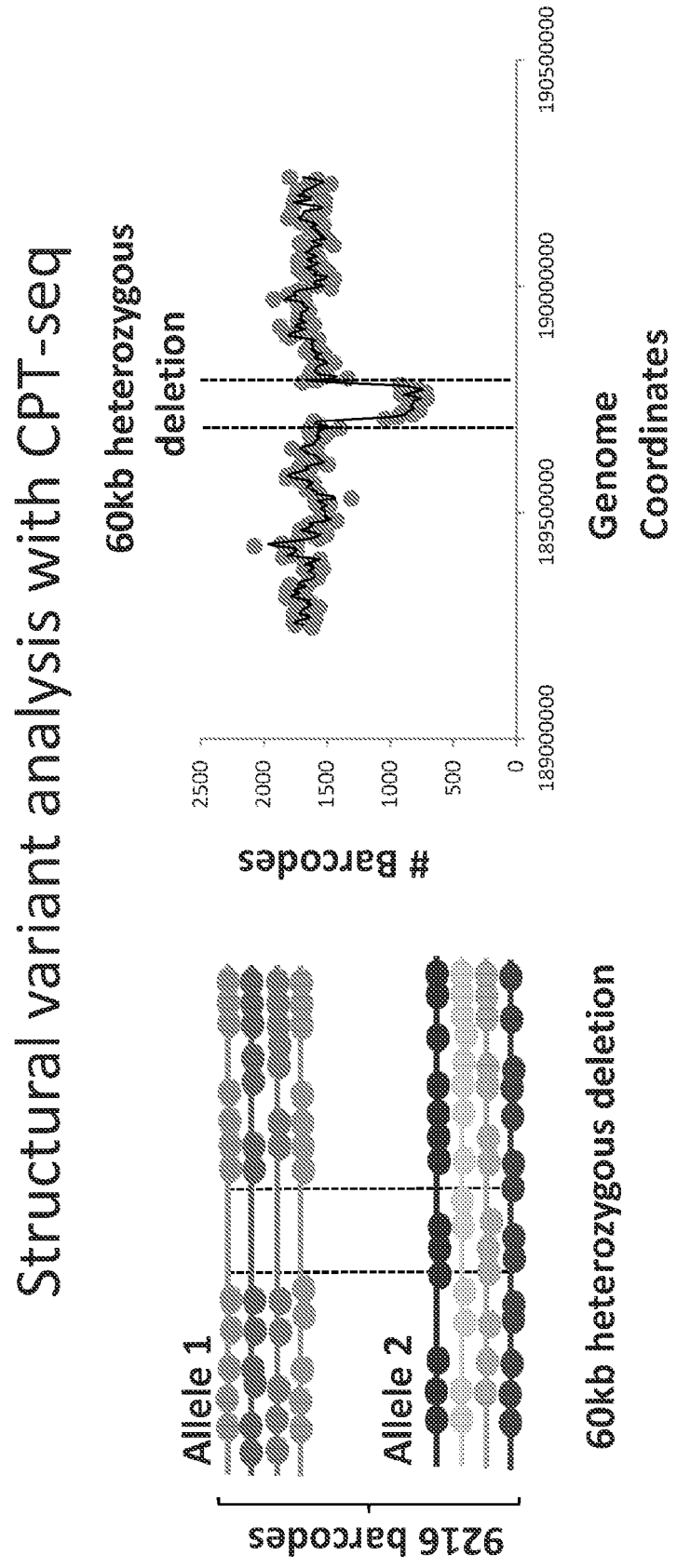

Structural variant analysis with CPT-seq
60kb heterozygous deletion
Barcodes
2500
2000
1500
1000
500
0
189000000
189500000
190000000
190500000
Genome Coordinates
9216 barcodes
Allele 1
Allele 2
60kb heterozygous deletion
Heterozygous 60kb deletion on Chr 1 detection with CPT-seq.

Fig. 47A

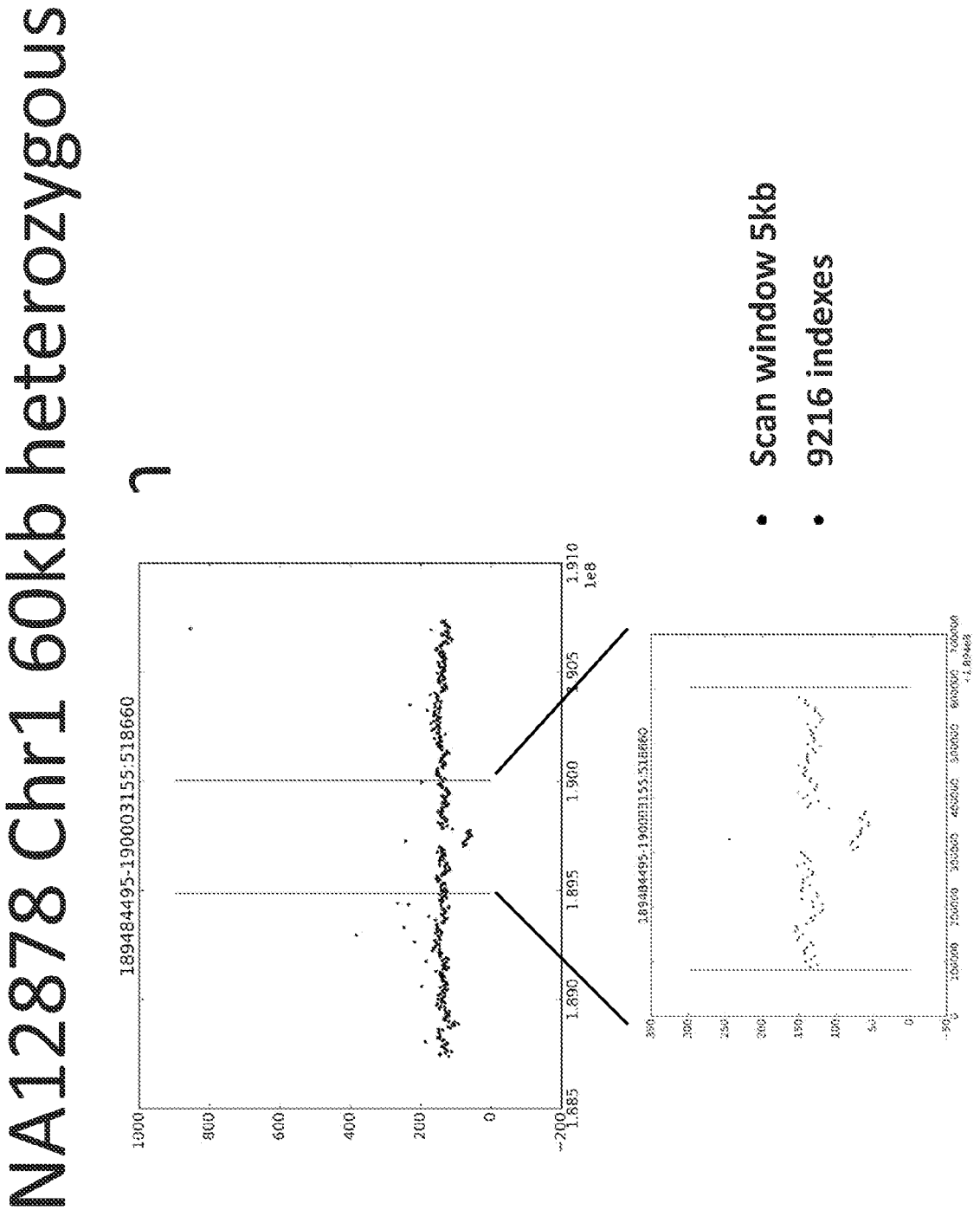
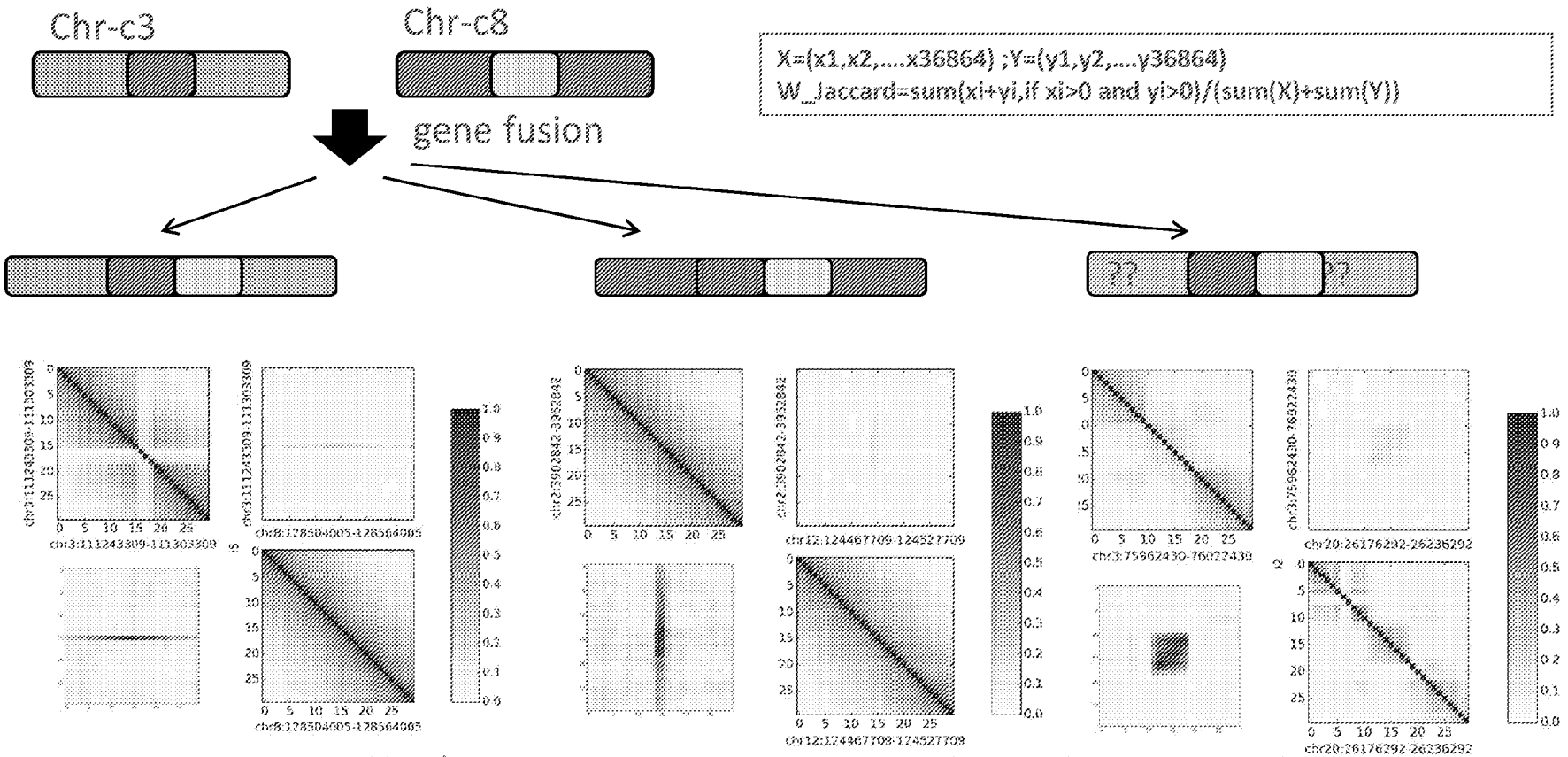

NA12878 Chr1 60kb heterozygous
189484495-190003155:518660
1000
800
600
400
200
0
-200
1.885
1.890
1.895
1.900
1.905
1.910
1e8
Scan window 5kb
9216 indexes

Fig. 47B

Gene fusion detection with CPT-seq



- Scan window is 2kb w/ each window a 36864 vector in which each element records how many reads have been found in the window.
- For each window pair (X,Y), the weighted Jaccard index is calculated and displayed as a heatmap.

Fig. 48

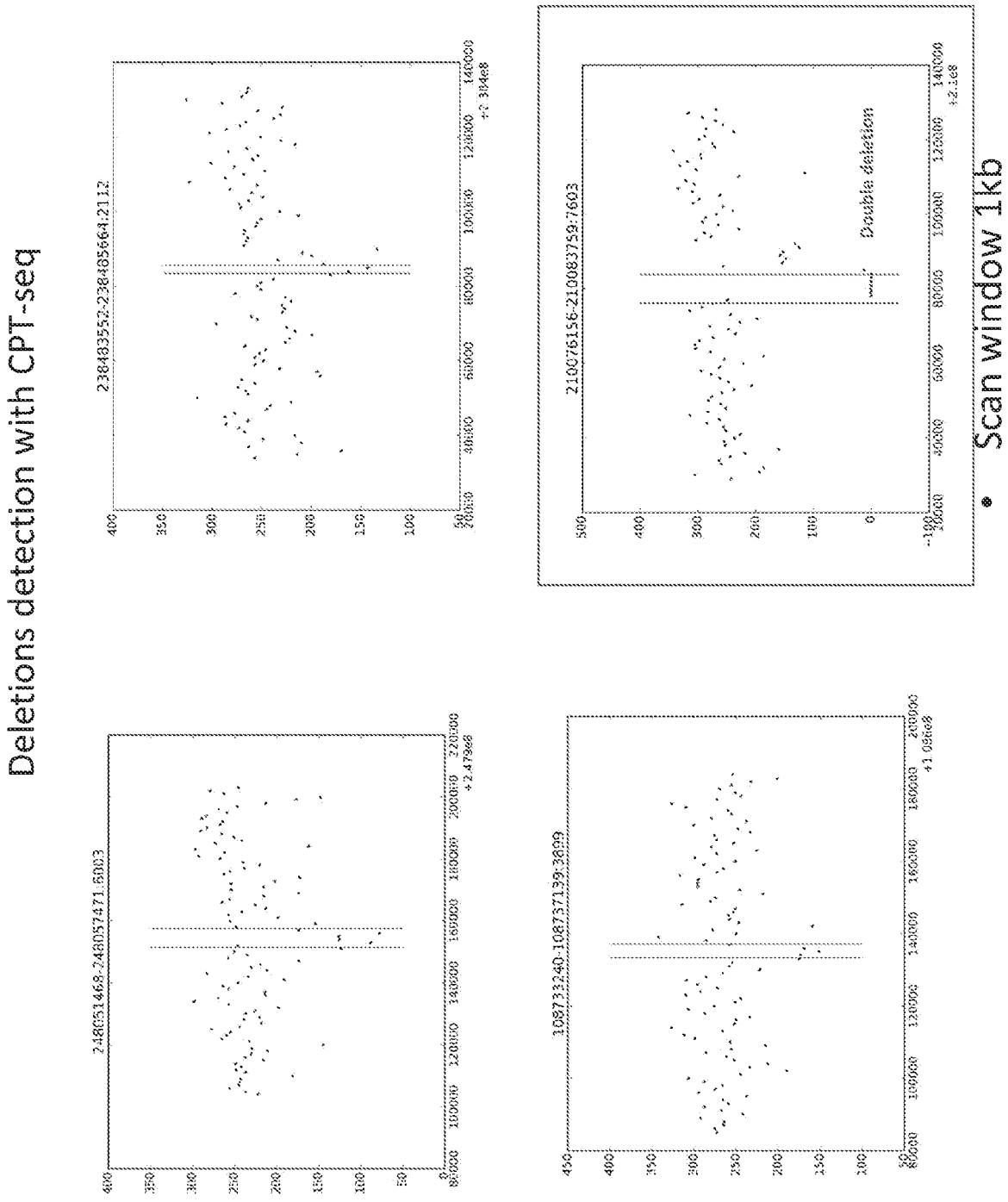

Deletions detection with CPT-seq
238483552-238485664:2112
248051468-248057471:6003
108733240-108737139:3899
210076156-210083759:7603
Double deletion
Scan window 1kb

Fig. 49

Bisulfite Conversion Efficiency Optimization

- Optimized Promega's MethylEdge Bisulfite Conversion system to improve conversion efficiency.

Cond	DNA	Beads	BSC Treatment
1	10ng	No	1hr @ 60°C / 0.3M NaOH
2		Yes	1hr @ 60°C / 0.3M NaOH
3			1hr @ 60°C / 1M NaOH
4			1hr @ 65°C / 0.3M NaOH

- ME sequence analyzed to determine efficiency of bisulfite conversion treatments.

Unconverted ME	GATGTGTATAAGAGACAG
BSC ME	AATATATATAAAAAACAA

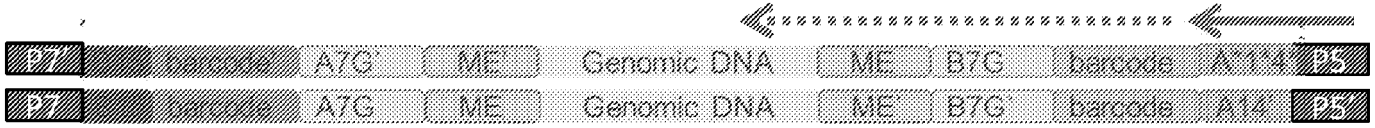


Fig. 50

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Bisulfite Conversion Efficiency Optimization

Results

- 95% bisulfite conversion (BSC) of indexed-linked libraries attached to beads.
- Similar PCR yields observed between bisulfite conditions > Harsher bisulfite treatment did not appear to degrade libraries.

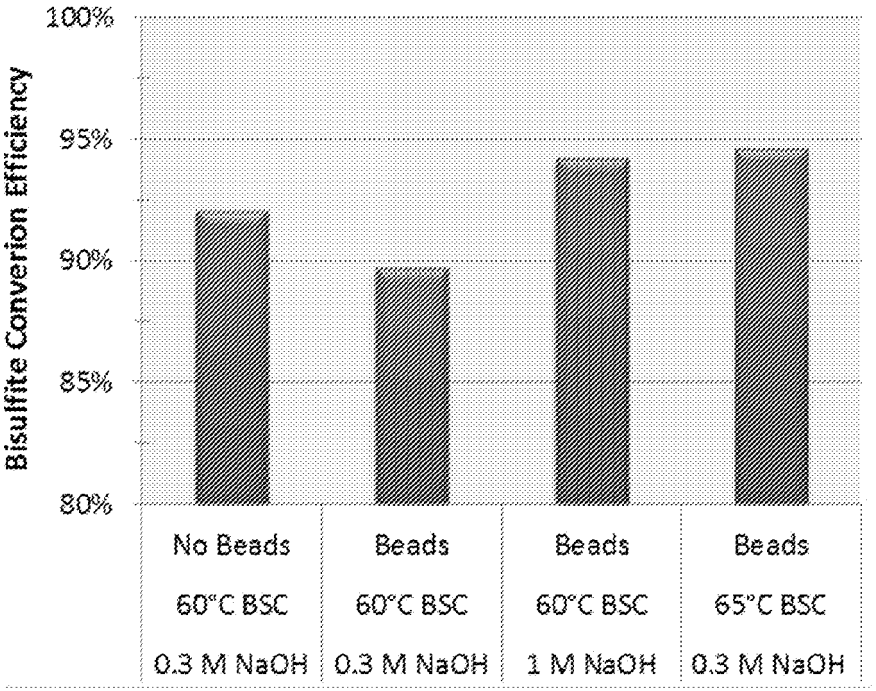


Fig. 51

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IVC plot (intensity versus cycles per individual base)



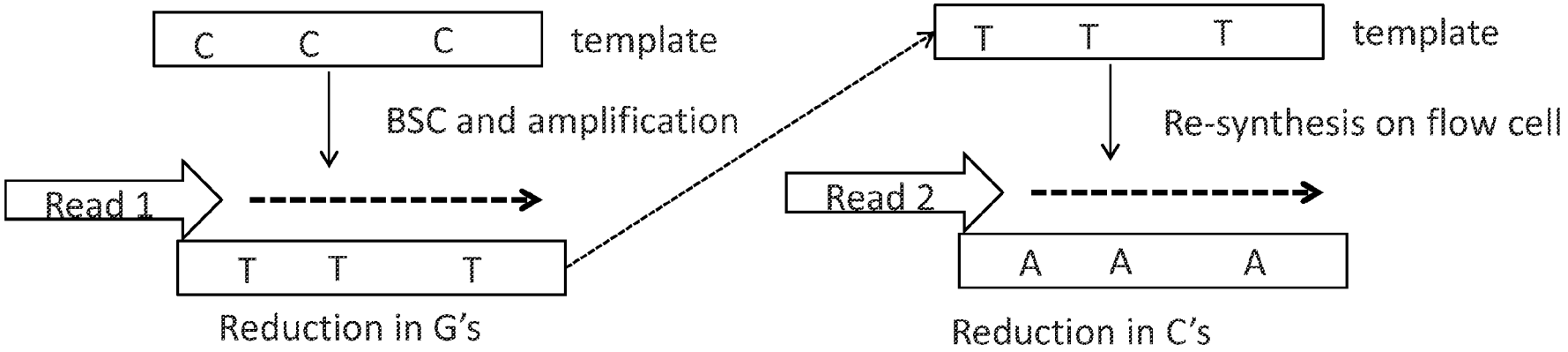
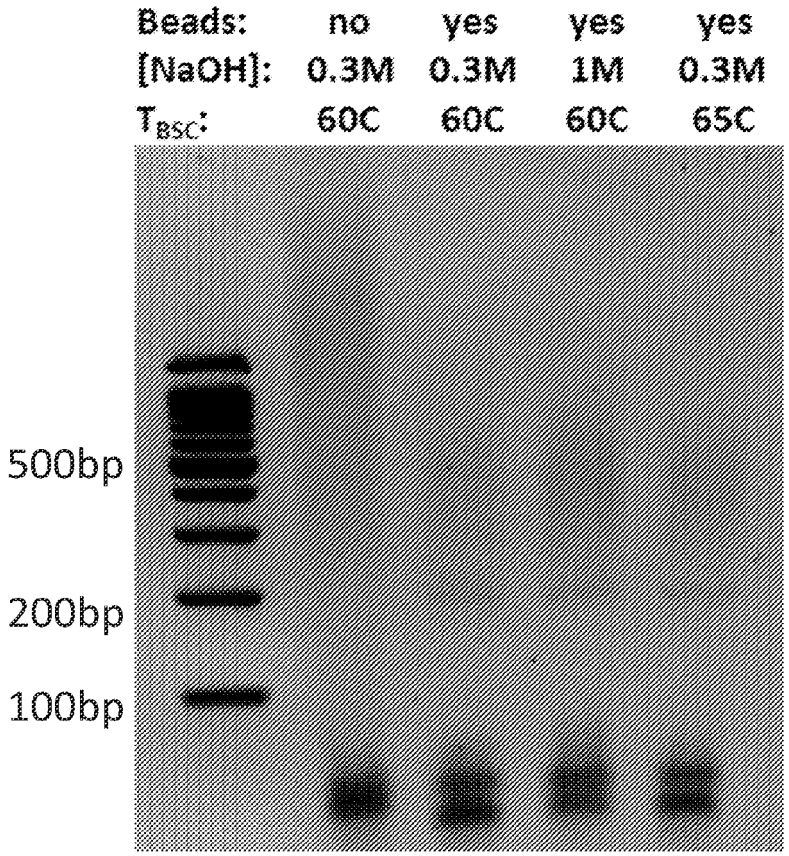


Fig. 52

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Bisulfite Conversion Efficiency Optimization



-Indexed-linked libraries after bisulfite conversion.
-Efficiency of bisulfite conversion ~95%

Fig. 53

Enrichment of CPT-Seq libraries

Cond	Library	Probe Set	[Probe] pM/ea	Hyb Volume	Hyb Ramp	EHB	Elution	Washex	SMB
1	1.1ug CPTSeq	DNA Dunn A, B, K	100pM	25uL	95 > 58 (O/N)	Form, Nxt Blkrs	ET1	Std	3x
2	1.1ug CPTSeq	EEX	2.5uL	25uL	95 > 58 (O/N)	Form, Nxt Blkrs	ET1	Std	3x
3	1.1ug CPTSeq	RNA Dunn A, B, K	100pM	25uL	95 > 65 (O/N)	Form, Nxt Blkrs	RNase H	Std	3x

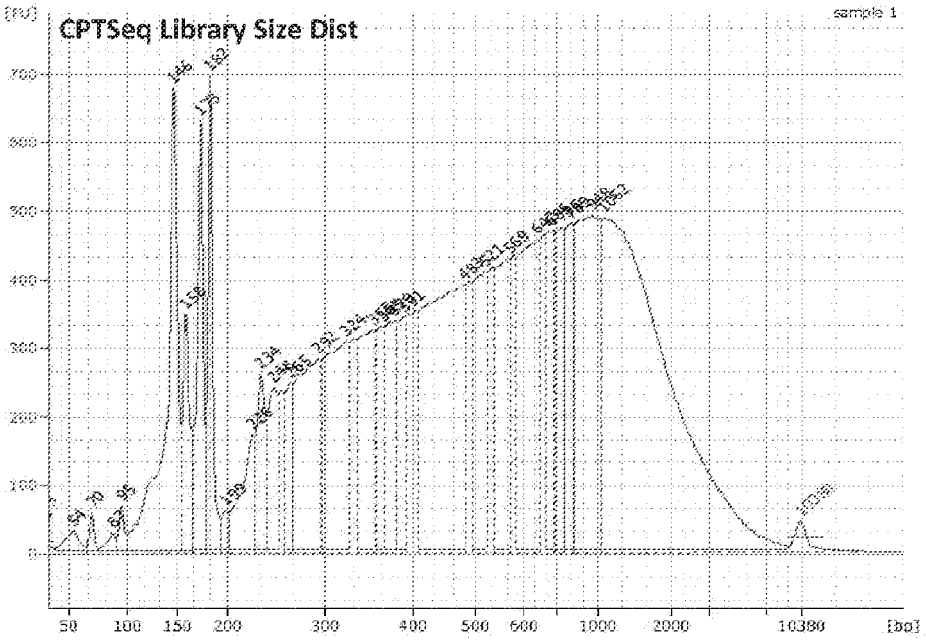


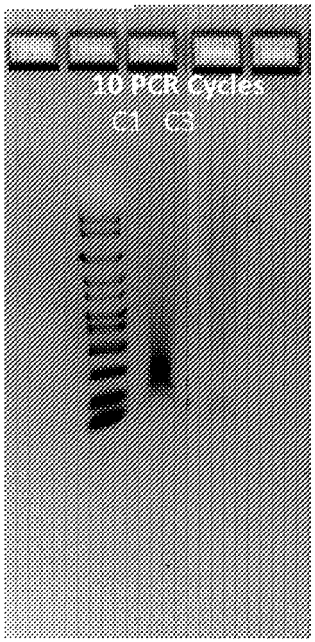
Fig. 54

CPT-Seq Library

≈ 1ug/library prep

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CPT-Seq Libraries Post Enrichment



- Targeted indexed-linked sequencing libraries

Fig. 55

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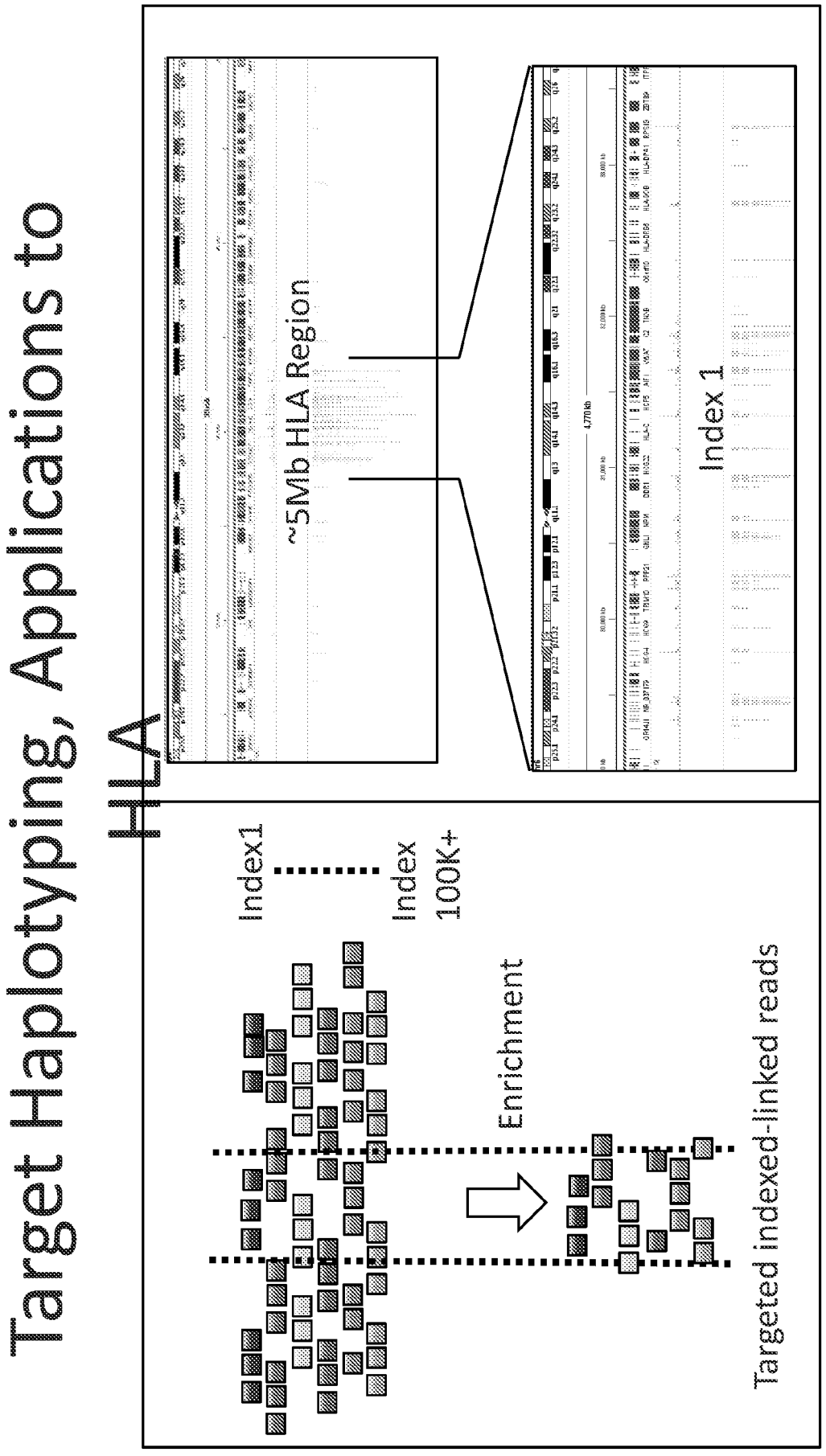

Target Haplotyping, Applications to HLA
~5Mb HLA Region
Index 1
Index1
Index
100K+
Enrichment
Targeted indexed-linked reads

Fig. 56

Mechanisms for Swapping
1 - DNA binds to two differently indexed beads
– Possible solutions:
• More space between beads (dilute DNA more before adding)
• Add dummy beads to space complex-beads out more
2 - Complexes come apart and re-form
– Possible solutions:
• Be more gentle with mixing steps
• Wash away monomers not bound by SA
• Crosslink?
• Form complexes fresh?
3 - SA-biotin bond breaks and complex re-hybs to another bead
– Possible solutions:
• Trap biotin beads
• Block free streptavidin sites so that biotinylated oligos can't bind
4 - Contamination
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Figure 57

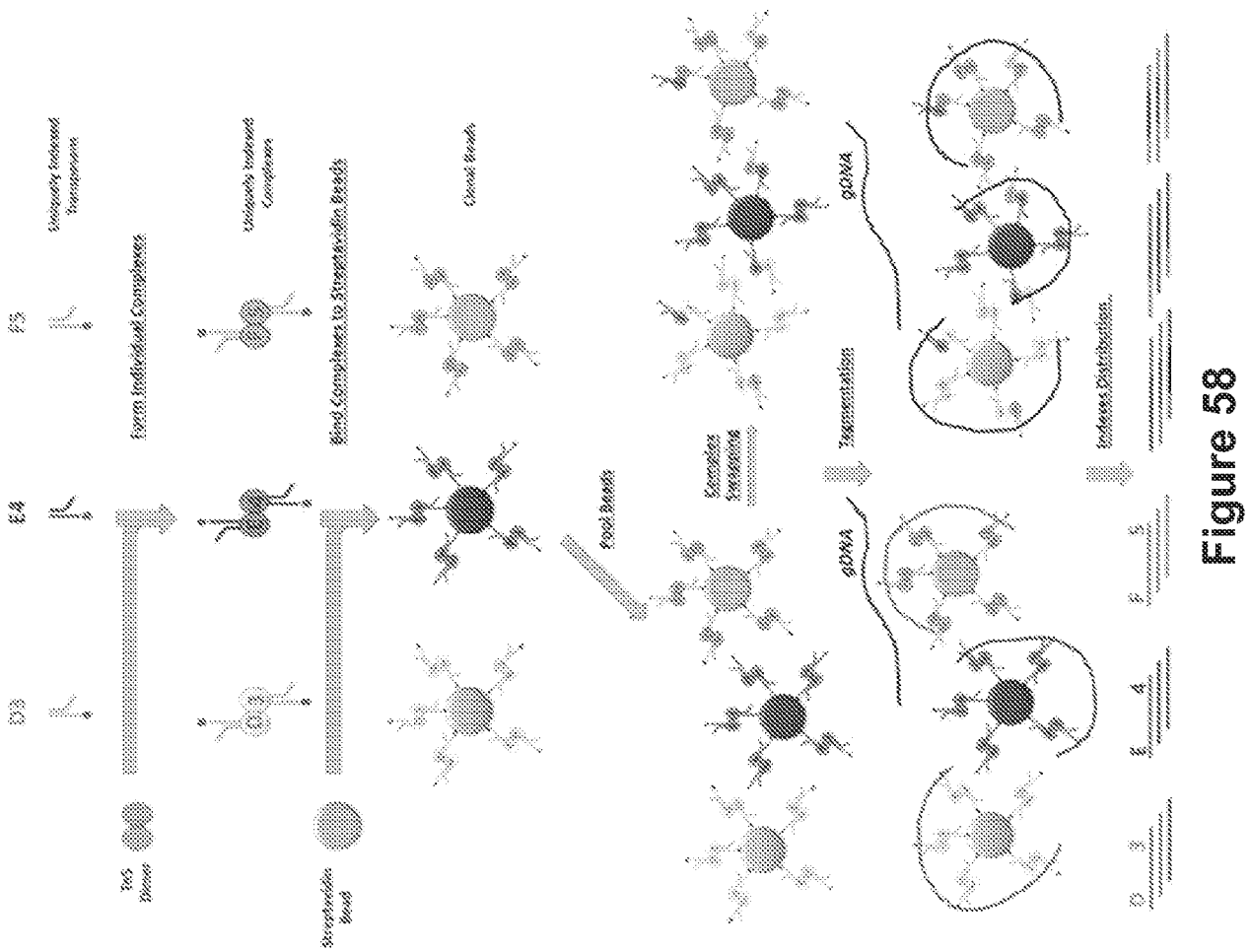

D3
E4
E5
Form Individual Complexes
Uniquely Indexed Complexes
Uniquely Indexed Transposomes
Bind Complexes to Streptavidin Beads
Streptavidin Bead
Bead Beads
Pool Beads
Complex Fragments
gDNA
Fragmentation
gDNA
Indexed Distribution

Figure 58

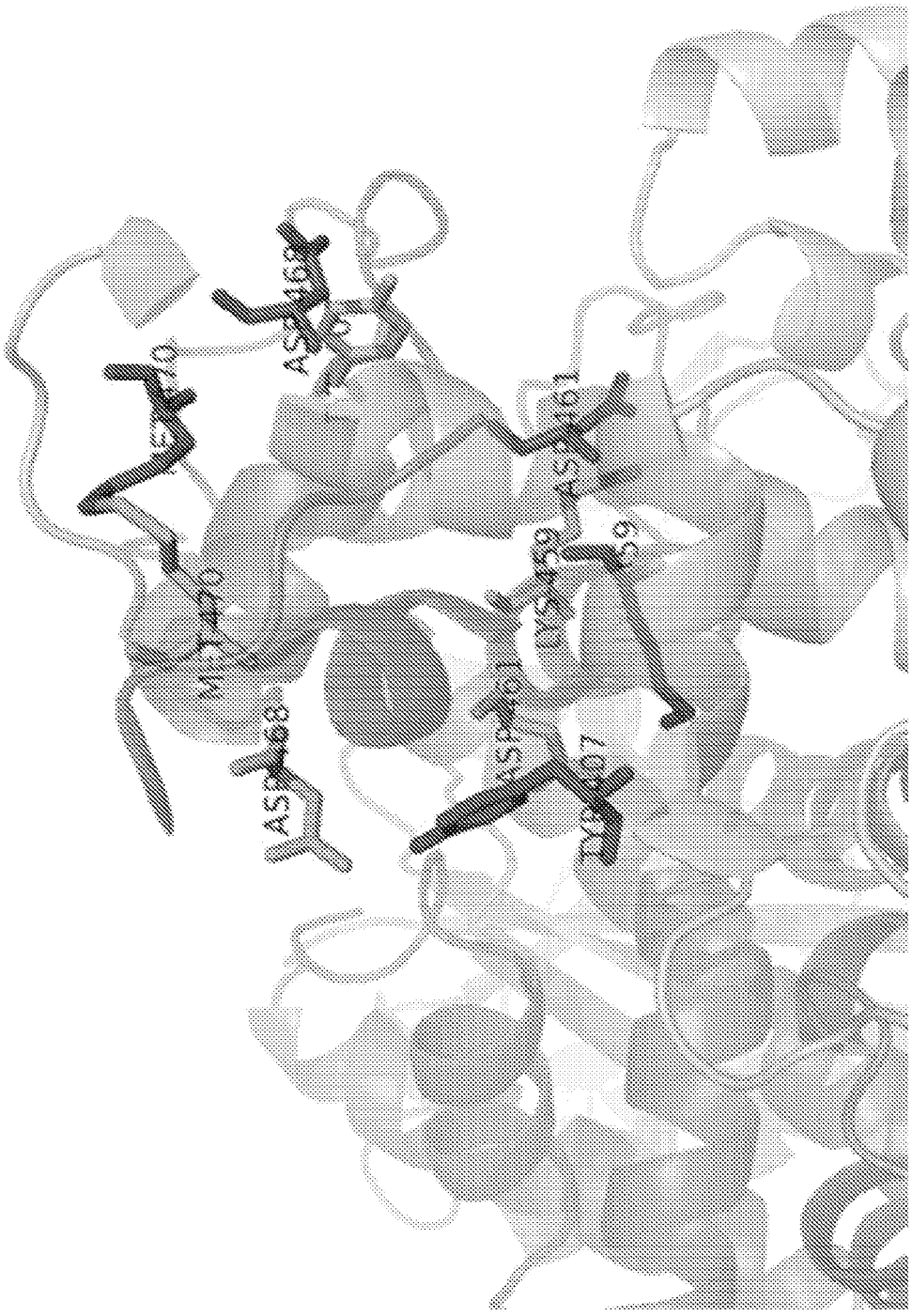

GLU470
ASP468
MET470
ASP468
HSP401
GLY459
ASP461
TYR407
459

Fig. 59

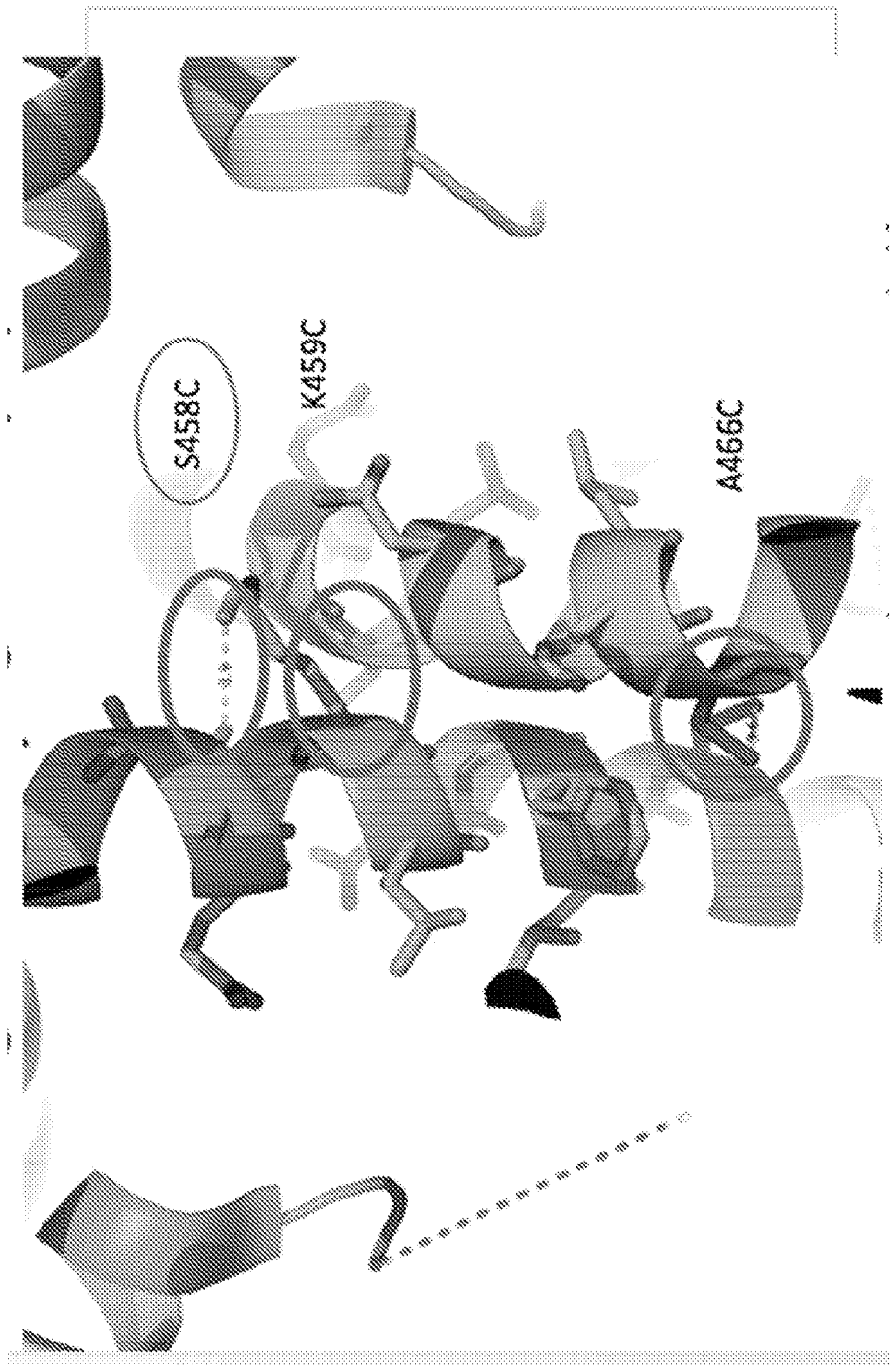


Fig. 60

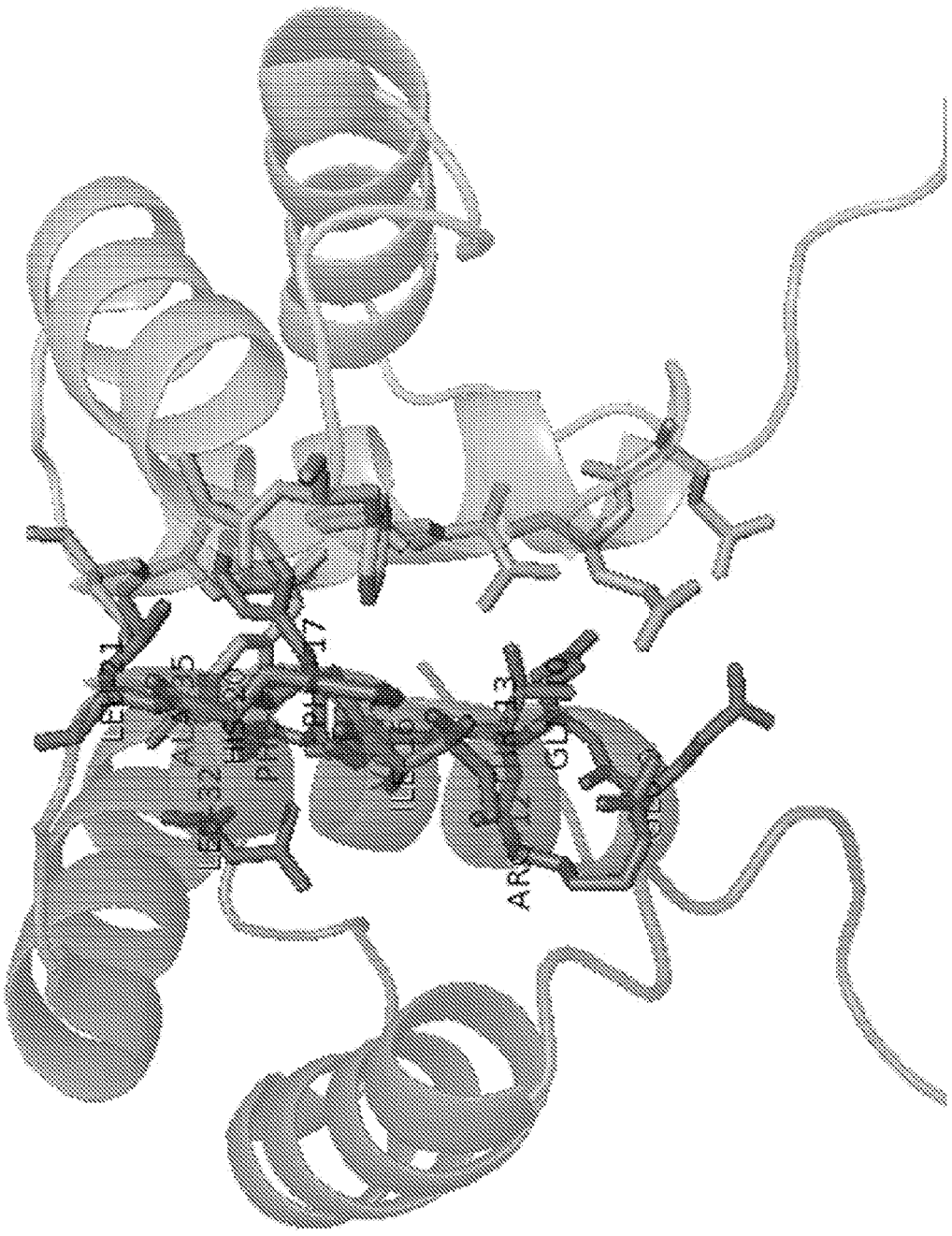


Fig. 61

Fig 5. Dimer transposase nanoparticle bioconjugate (dTnp-NP)

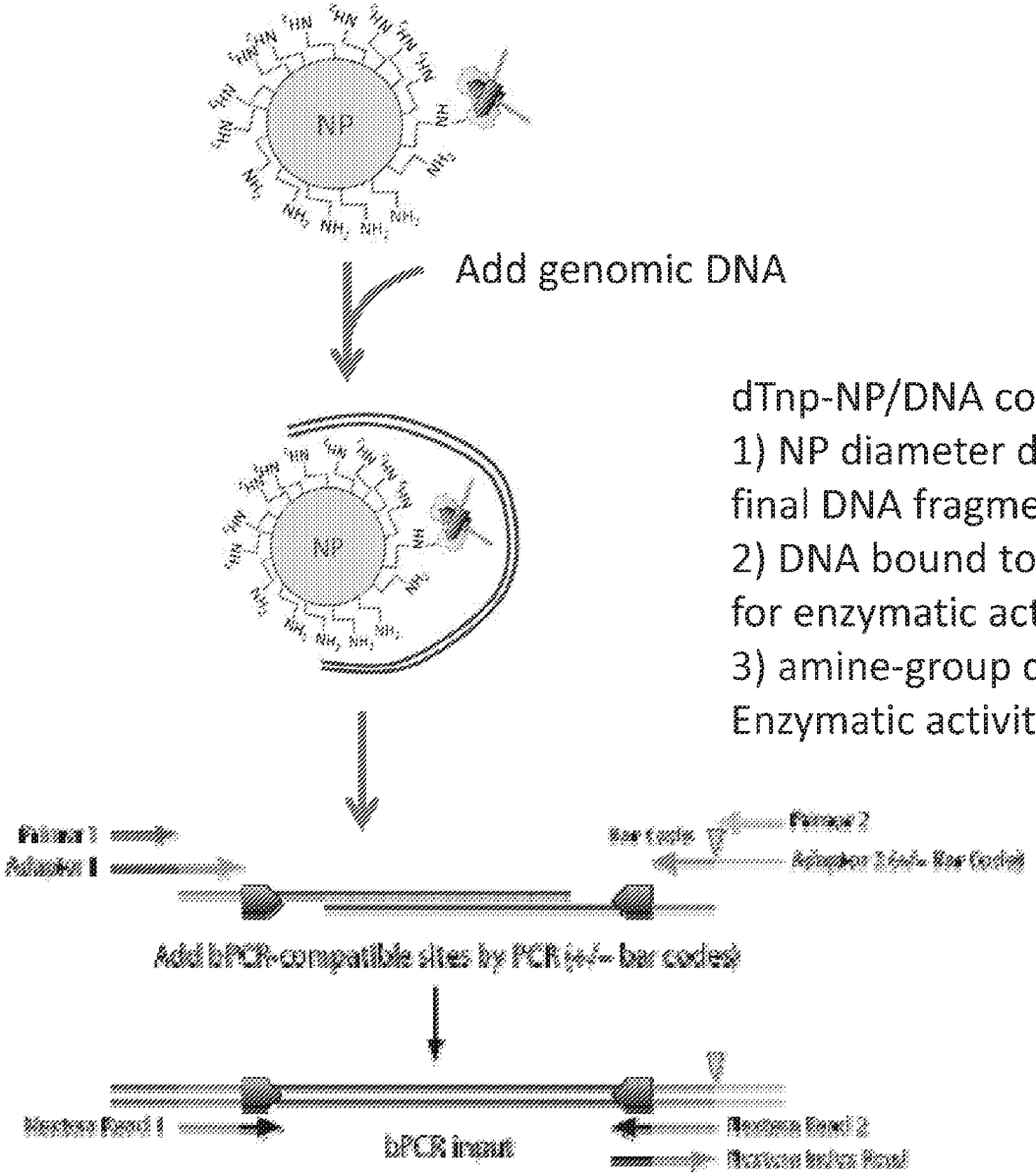


Figure 62

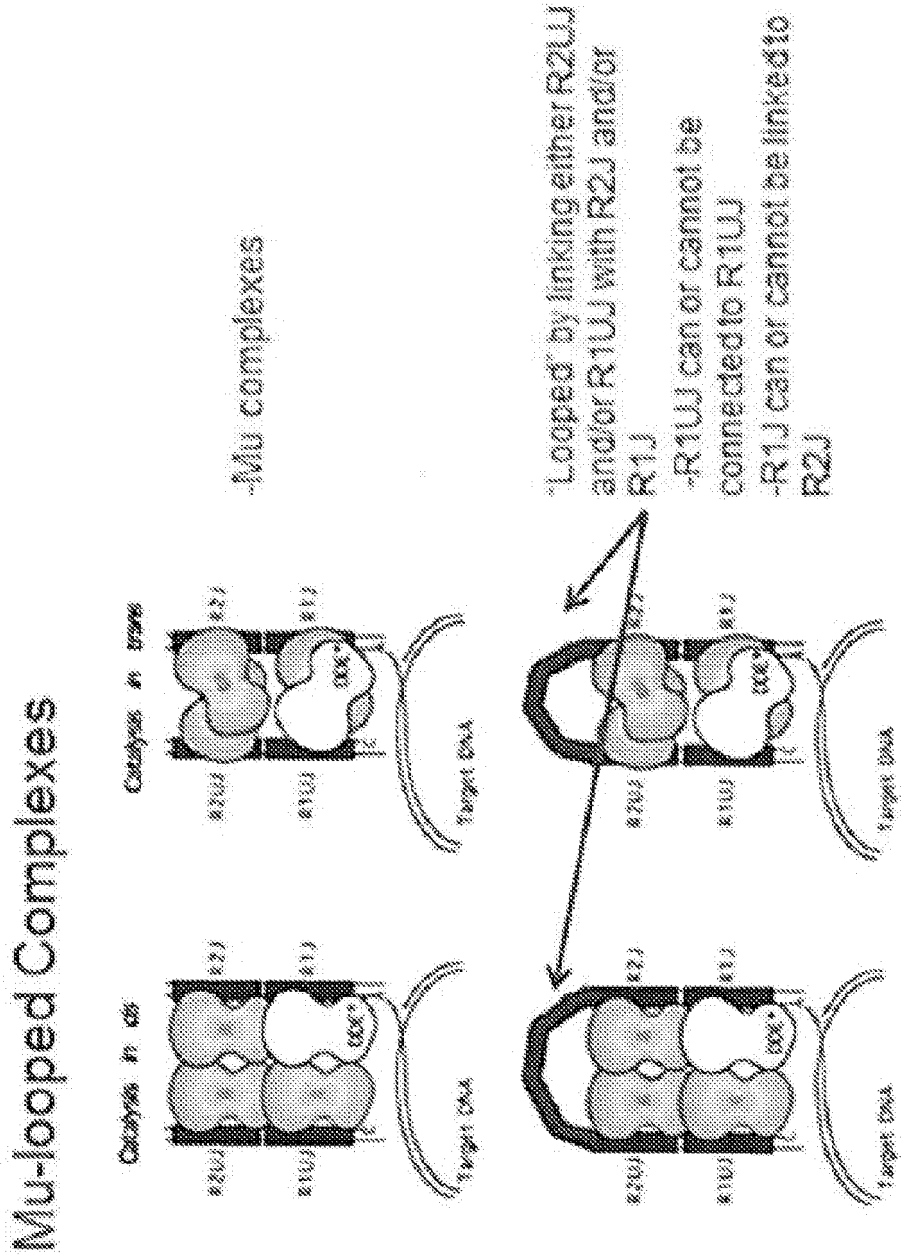

Mu-looped Complexes
Catalysis in cis
Catalysis in trans
-Mu complexes
"Looped" by linking either R2UJ and/or R1UJ with R2J and/or R1J
-R1UJ can or cannot be connected to R1UJ
-R1J can or cannot be linked to R2J
R2UJ
R1UJ
R2J
R1J
DDE*
Target DNA

Fig. 63

Indexed linked reads for assembly/phasing of pseudo-genes
UniqueB
Gene A
Gene A'
UniqueC
5kb
1kb
?
5kb
High molecular weight DNA
Lower molecular weight DNA
"Long" islands
"short" islands
Example, CYP2D6
Long islands (>30kb) of indexed linked reads will span the pseudogenes, gene A and A'
Due to the high sequence, it is not possible to determine which variant belongs to GeneA or GeneA'
Shorter islands will only link one variant in one of the pseudogenes with unique surrounding sequence.
-> This can be accomplished by shearing the DNA before CPT-seq or use combination of sheared and unsheared DNA

Fig. 64

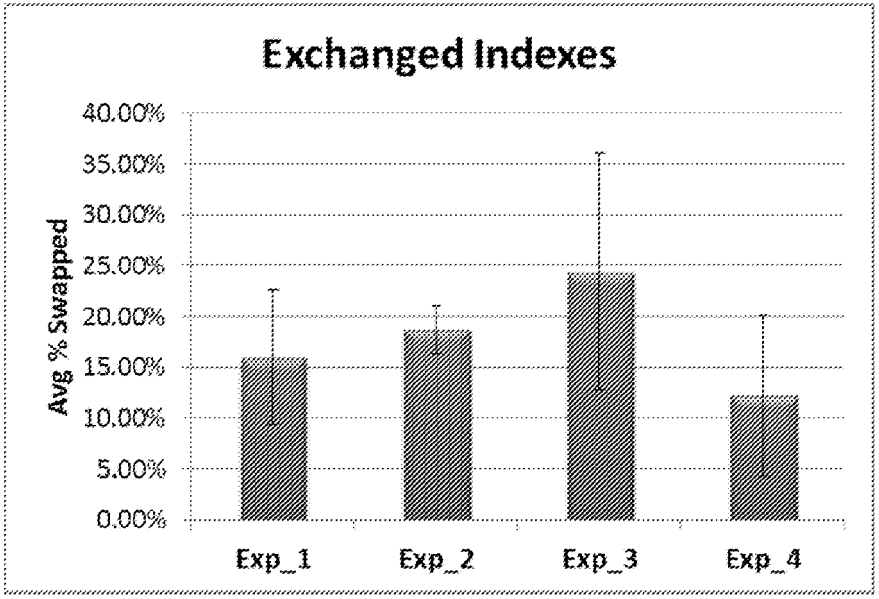


Figure 1: Index exchange. Bead with different indices were prepared. After mixing, Index exchange was determine by sequencing the libraries and reporting the indices for each library. % "swapped" was calculated as (D4+D5+E3+E5+f4)/(sum of all 96) and reported in the figure.

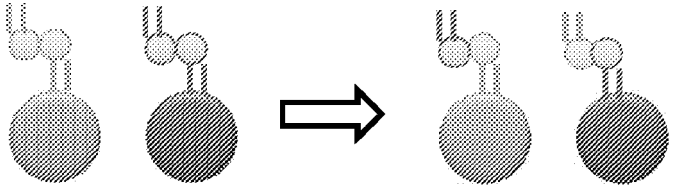


Fig. 65

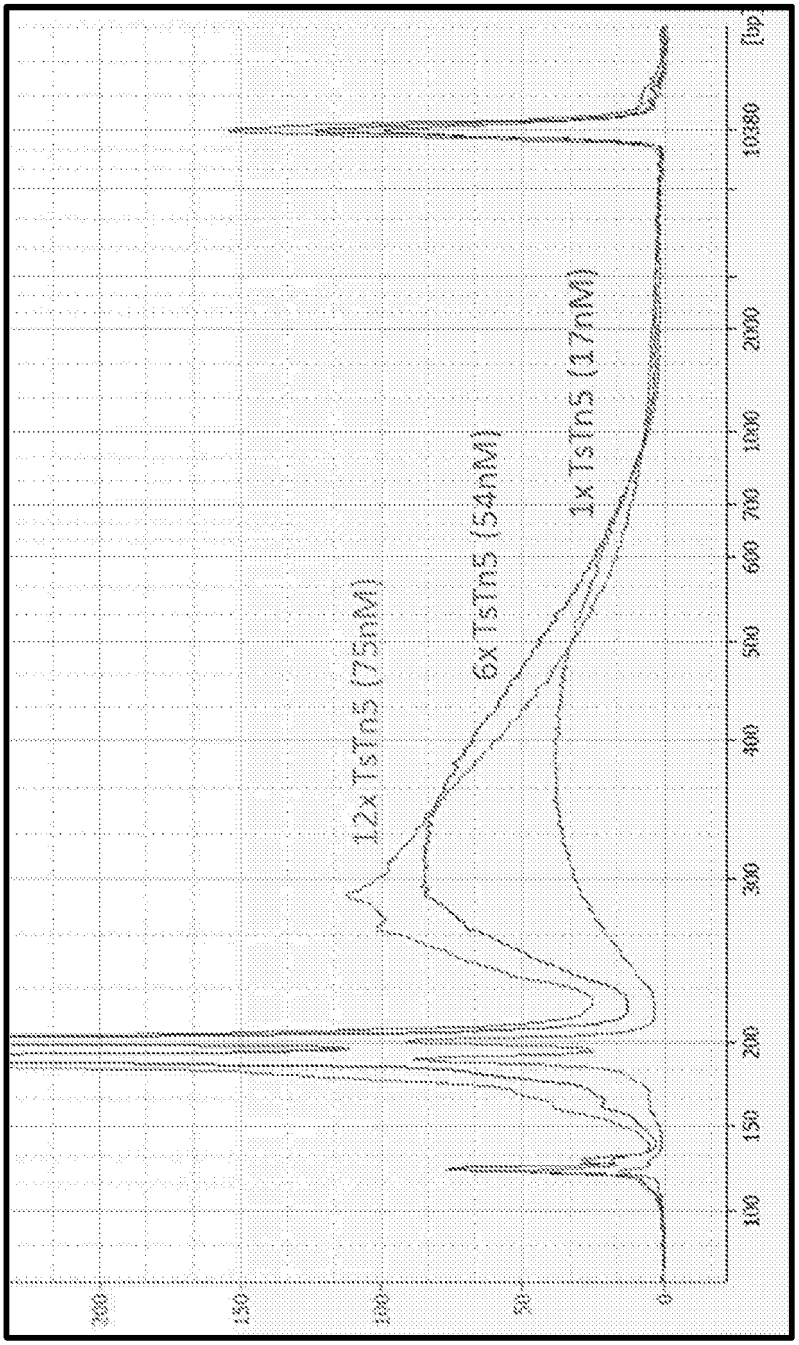

12x TsTn5 (75nM)
6x TsTn5 (54nM)
1x TsTn5 (17nM)
[bp]
10380
2000
1000
700
600
500
400
300
200
150
100
0
50
100
150
200

Fig. 66

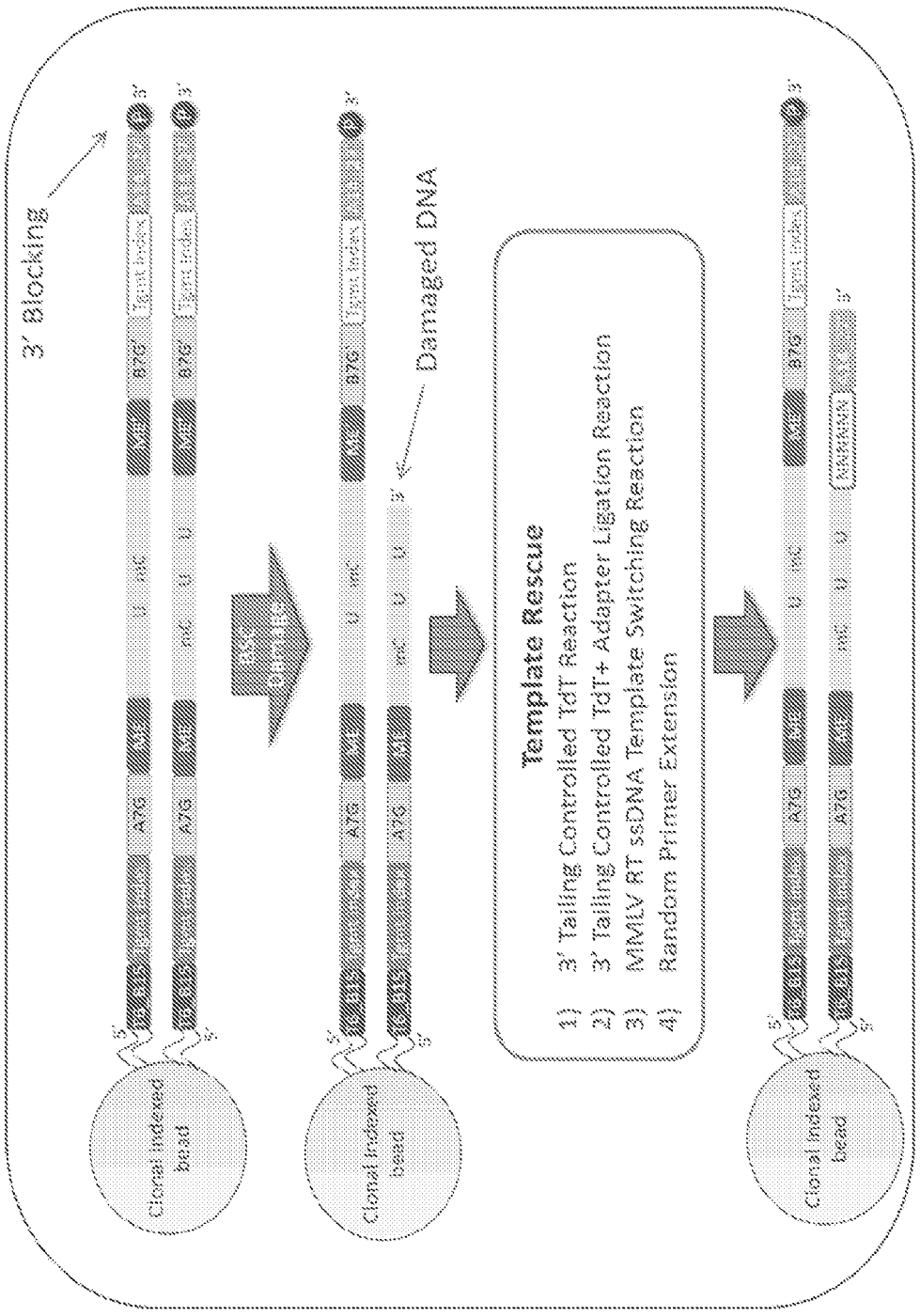

3' Blocking
Damaged DNA
Template Rescue
1) 3' Tailing Controlled TdT Reaction
2) 3' Tailing Controlled TdT+ Adapter Ligation Reaction
3) MMLV RT ssDNA Template Switching Reaction
4) Random Primer Extension
Clonal Indexed bead
Clonal Indexed bead
Clonal Indexed bead

Fig. 67

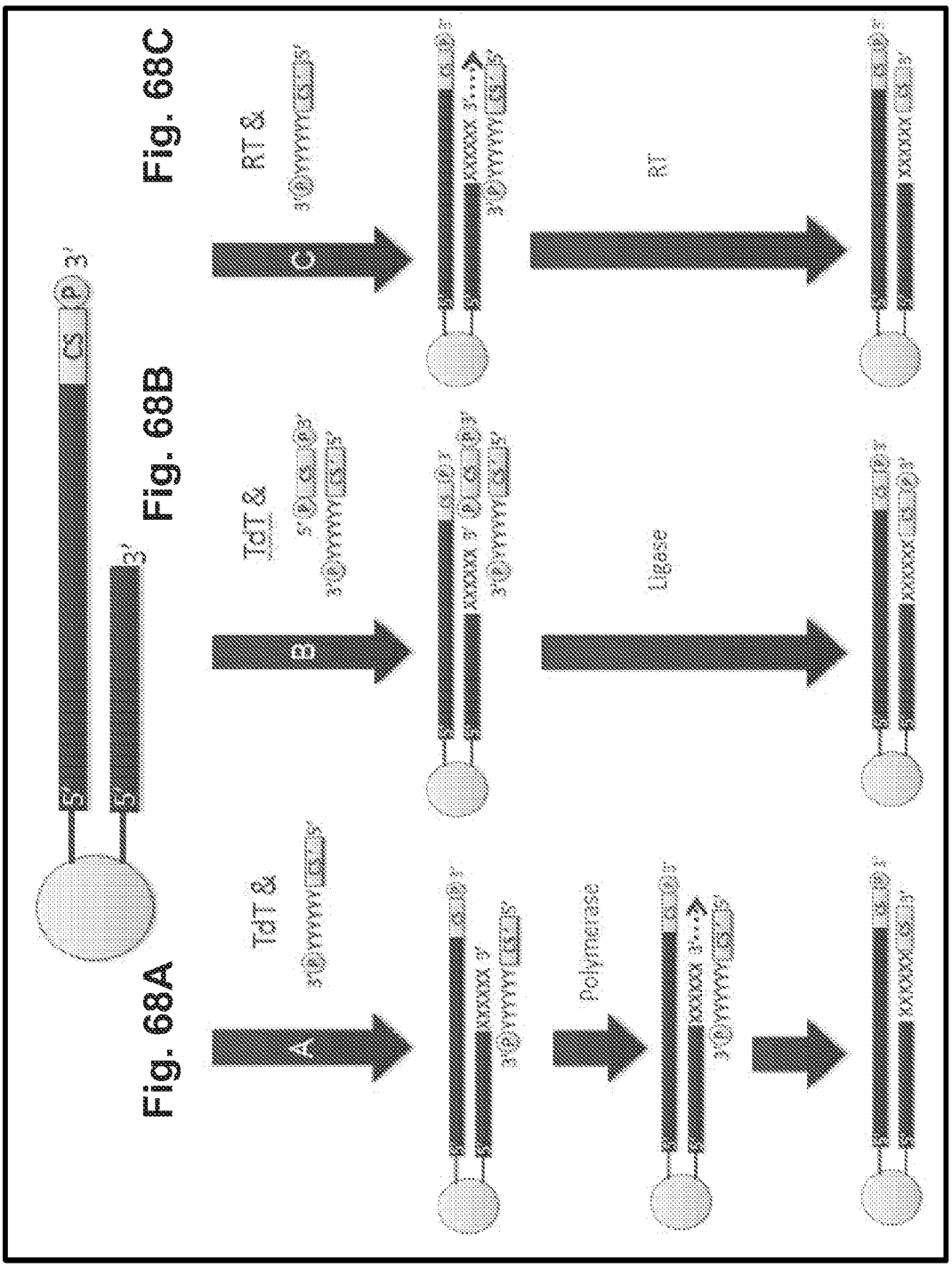


Fig. 68

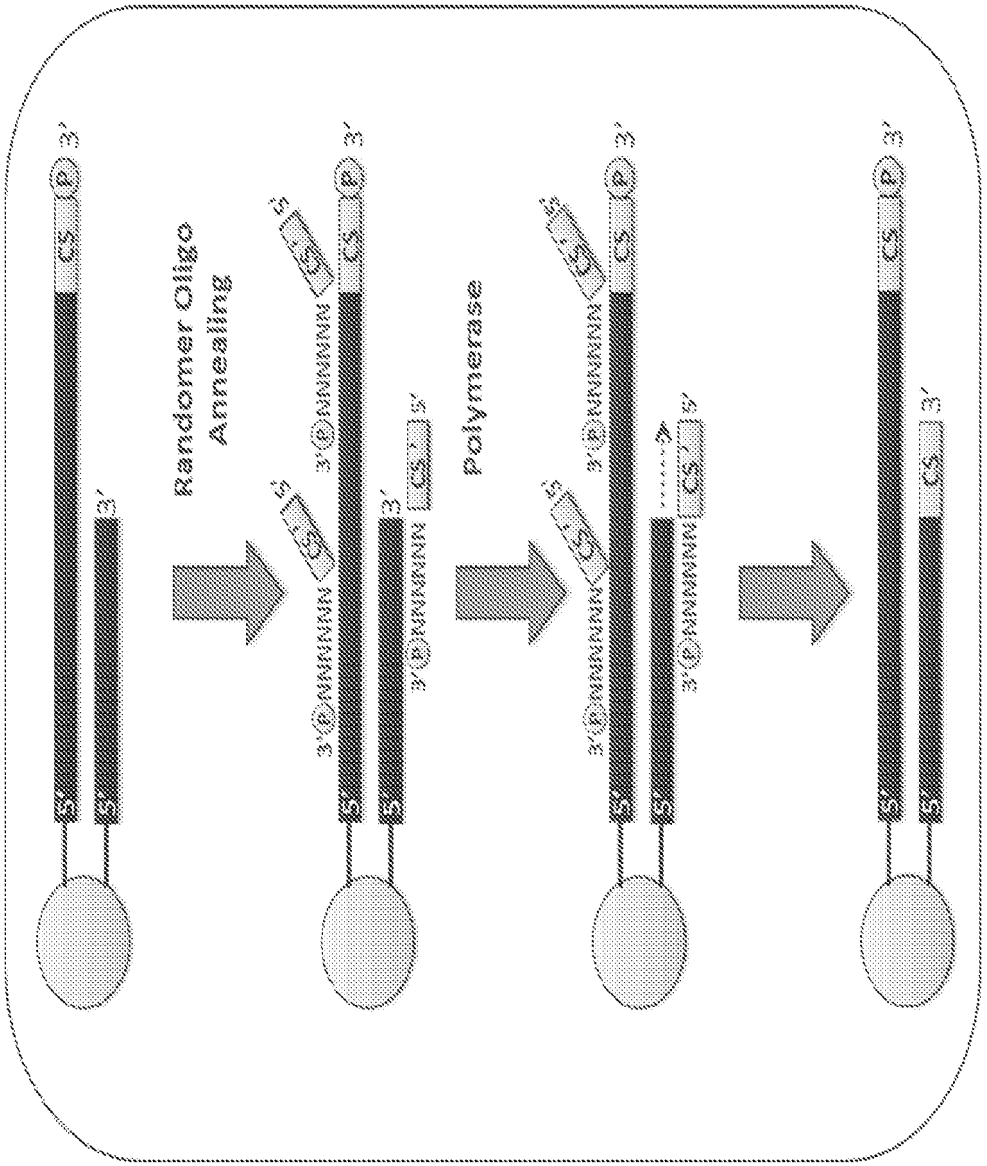


Fig. 69

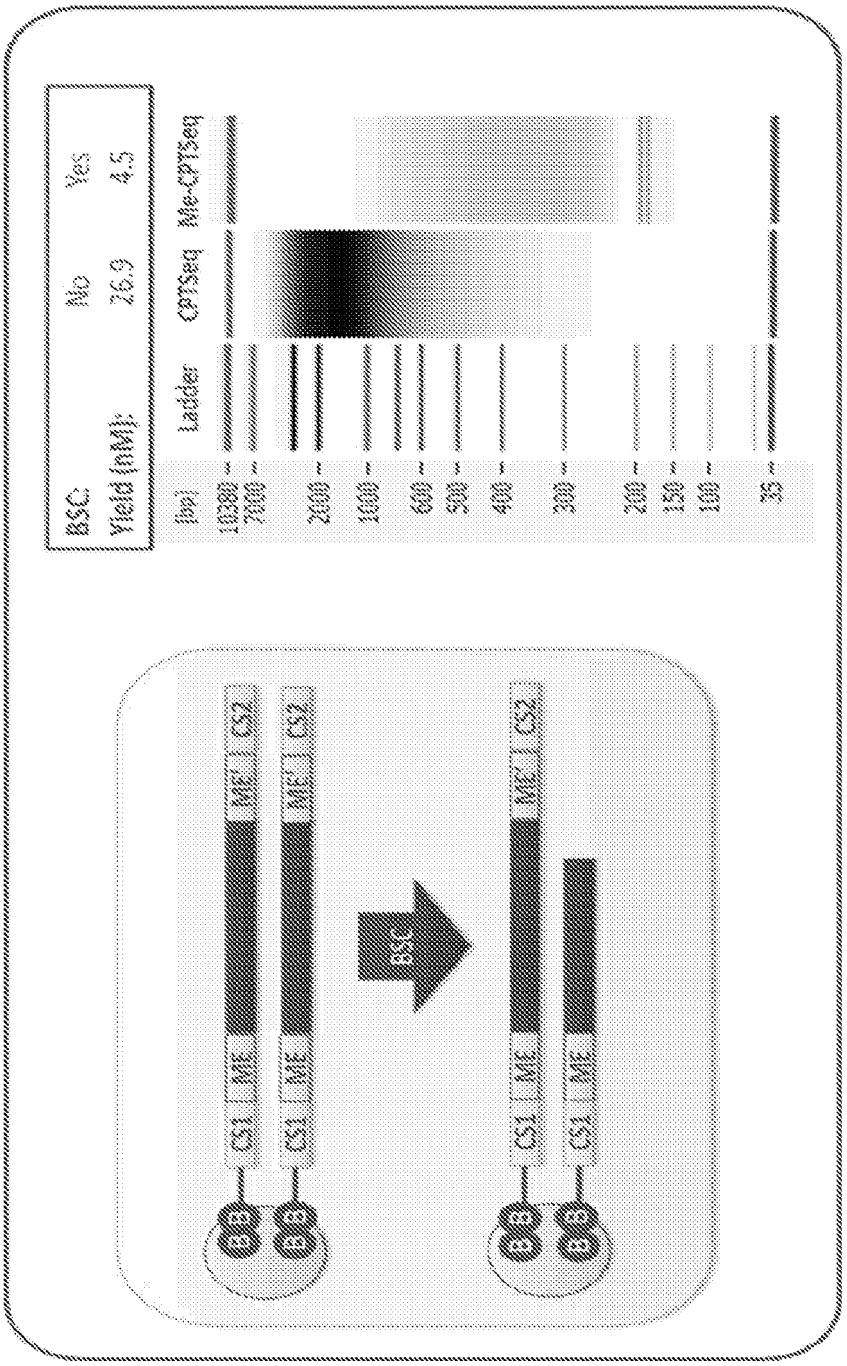

BSC
Yield (nM):
No
26.9
Yes
4.5
(bp)
Ladder
CPTSeq
Me-CPTSeq
10380
7000
2000
1000
600
500
400
300
200
150
100
35
CS1
ME
CS2
BB
BST

Fig. 70

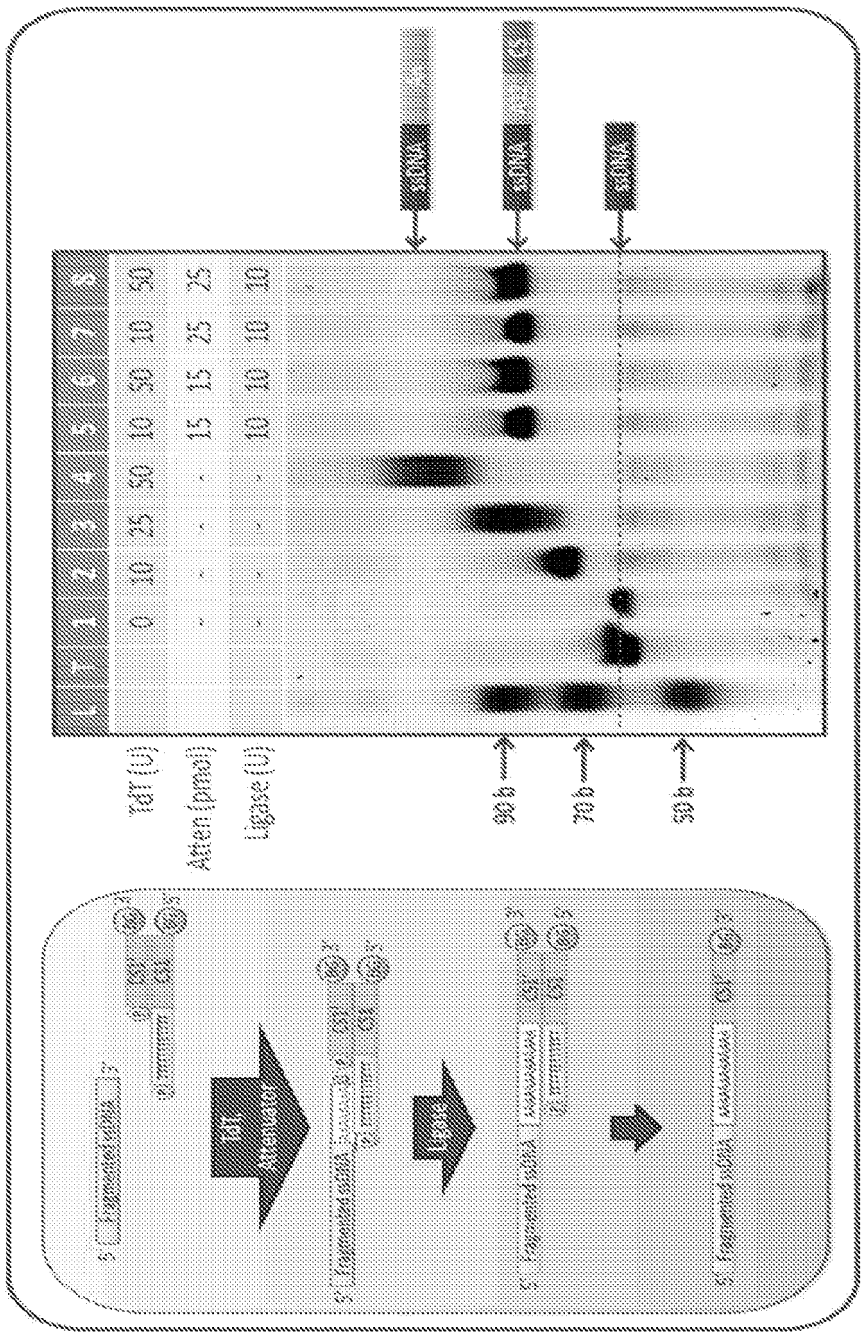

1 2 3 4 5 6 7 8
0 10 25 50 10 50 10 50
- - - - 15 15 25 25
- - - - 10 10 10 10
TdT (U)
Atten (pmol)
Ligase (U)
90 b
70 b
50 b
Attenuator
Ligate

Fig. 71

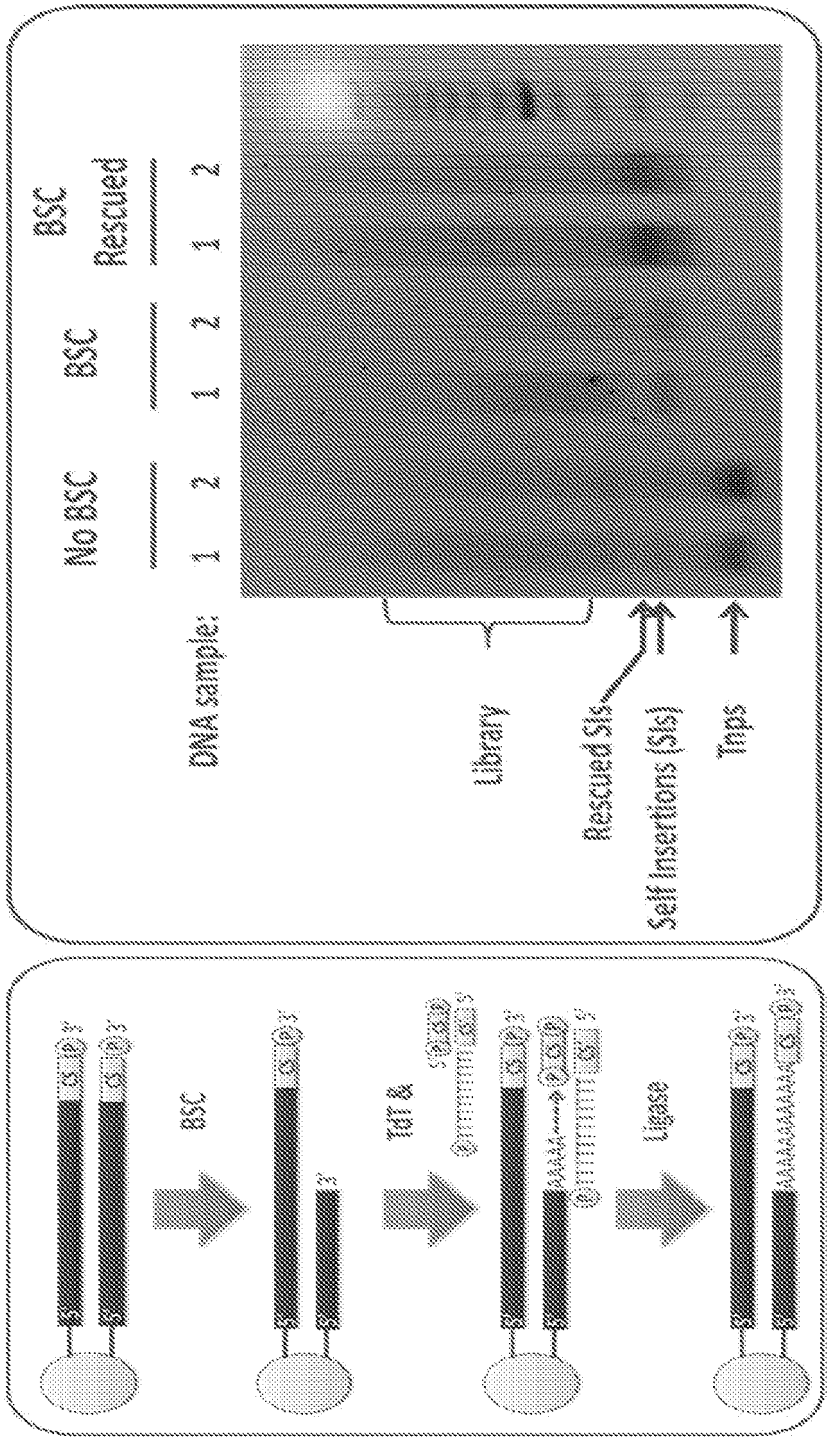

BSC
Tdt &
Ligase
DNA sample:
No BSC
BSC
BSC Rescued
1 2 1 2 1 2
Library
Rescued SIs
Self Insertions (SIs)
Tnps

Fig. 72

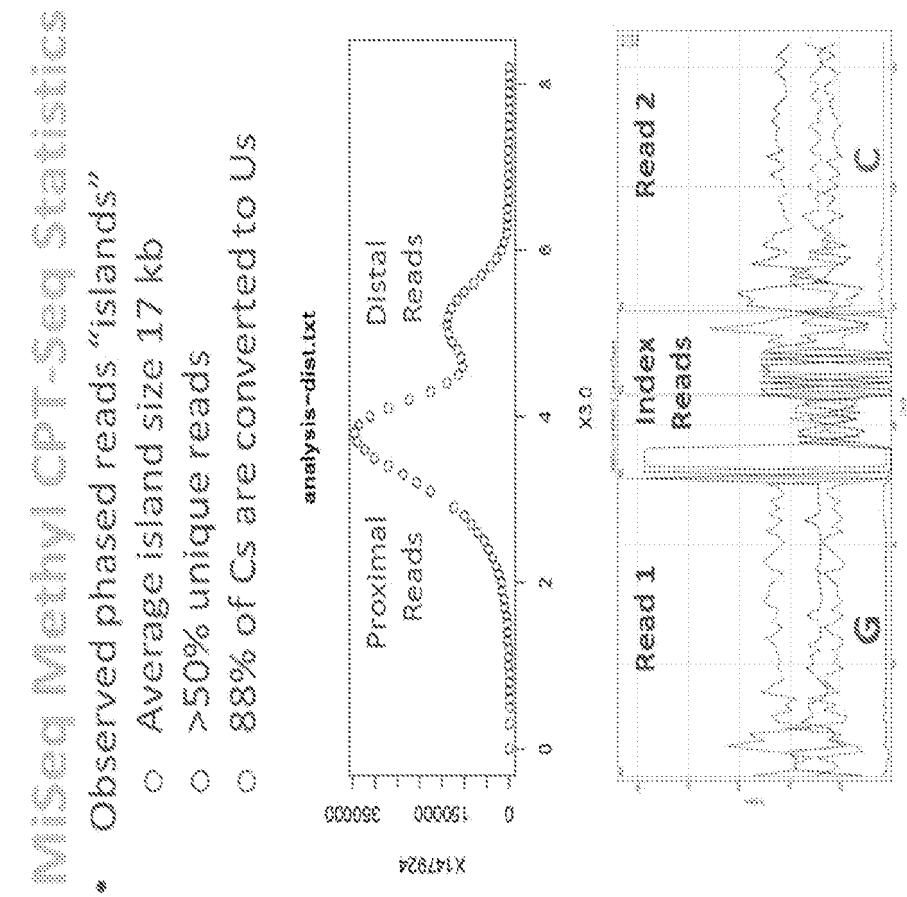

MiSeq Methyl CPT-Seq Statistics
Observed phased reads "islands"
Average island size 17 kb
>50% unique reads
88% of Cs are converted to Us
analysis-dist.txt
Proximal Reads
Distal Reads
X147924
0
150000
300000
0
2
4
6
8
X3.0
Read 1
Index Reads
Read 2
G
C

Fig. 73

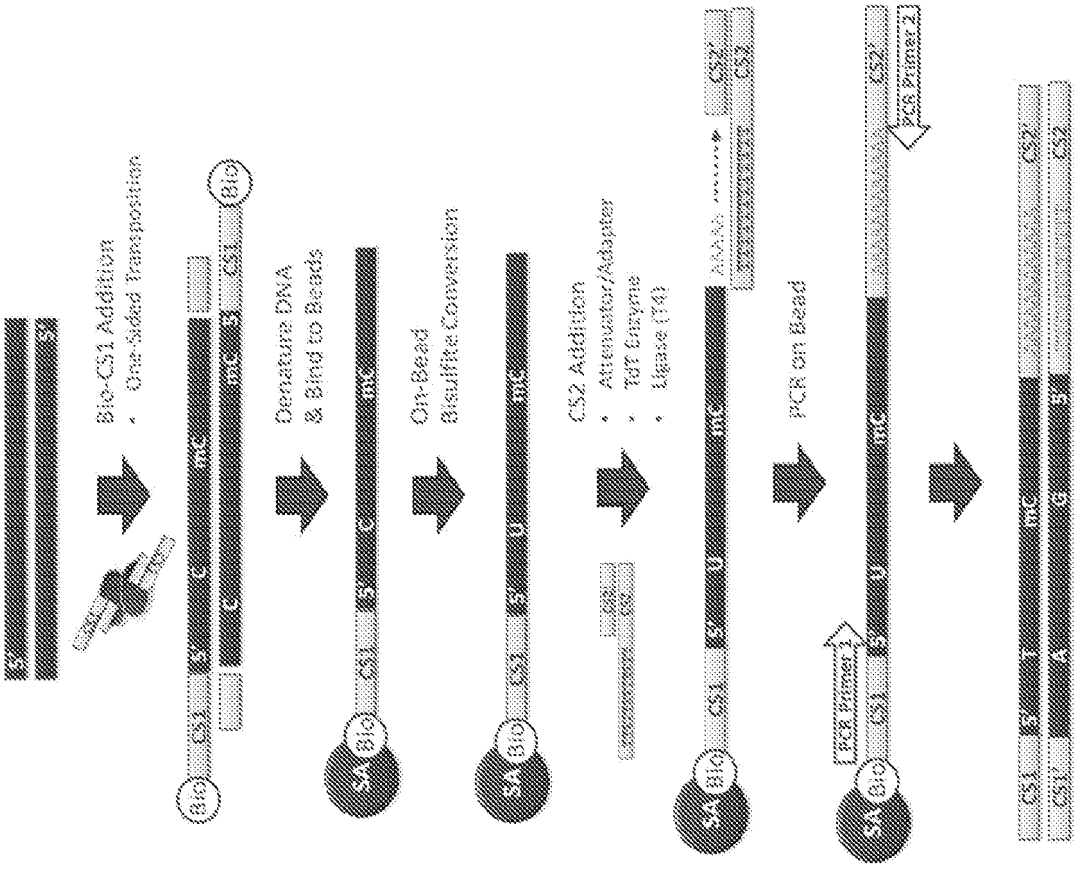

Bio-CS1 Addition
One-Sided Transposition
Denature DNA
& Bind to Beads
On-Bead
Bisulfite Conversion
CS2 Addition
Attenuator/Adapter
TdT Enzyme
Ligase (T4)
PCR on Bead
PCR Primer 1
PCR Primer 2
CS1
CS2
Bio
SA

Fig. 74

Fig. 75A

Fig. 75B

Fig. 75